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(57) Abstract

Plants are transformed with a DNA construct adapted to modify the expression of at least one senescence-related gene. Plants having modified senescence characteristics are subsequently selected. Specific senescence-related plant genes, their incorporation into DNA constructs and their use to inhibit or accelerate plant senescence are described.

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REGULATION OF SENESCENCE

This application relates to novel DNA constructs, plant cells containing such constructs and plants derived therefrom. In particular, it relates to the modification of the senescence process in plants.

The process of plant senescence has been well-studied (see for example, Plant Senescence: Its Biochemistry and Physiology, eds. Thompson et al, 1987). Senescence is a controlled series of biochemical and physiological events comprising the final stage of development. The changes taking place in senescence form a genetically programmed sequence, with close co-ordination at the cell and tissue level. Cells remain viable and show tight metabolic regulation until the end of senescence. Senescence may be caused by a variety of external factors (including light, temperature, water/minerals, pathogens) or internal factors (including space, light, nutrients, flowering/pollination, growth substances). A genetic switch is triggered which modifies gene expression at the transcriptional and/or post-transcriptional level and induces a change in cell/tissue function resulting in senescence. For example, during leaf senescence, the photosynthetic apparatus is dismantled and leaf function changes from carbon assimilation to nitrogen/phosphorus mobilisation. Senescence involves pigment degradation, proteolysis and nucleic acid breakdown leading to nitrogen redistribution and phosphorus remobilisation to other plant parts. It also involves the respiration of lipids and carbohydrates.

In an agricultural context, leaf expansion and senescence are quantified as leaf area index and leaf area duration, and these factors are known to be major determinants of yield in many crops (Thomas, 1992 in Crop Photosynthesis: Spatial and Temporal Determinants, Baker and Thomas (eds), Elsevier, pages 11-41). The importance of delayed senescence in increasing the yield of determinate crops has been confirmed by studies on several species (for example, Tollenaar and Daynard, 1978, Plant Sci, 78:199-206; Thomas, 1987 in Developmental Mutants in Higher Plants, Thomas and Grierson (eds), Cambridge Univ Press, pages 245-265).

Genetic variation exists for senescence and has, usually incidentally or empirically, been exploited for crop improvement by traditional plant breeding. Variants showing delayed or inoperative senescence are known in a range of species such as maize, sorghum, oats, rice, wheat, soybean, faba bean, fescue, fruit crops and trees. For example, genetic variation exists for symptoms of leaf senescence and genotypes with leaves which remain green for longer than normal are termed "stay-green" varieties. Stay-green variants are known in cereals, legumes, grasses and fruit species (Thomas and Smart, 1993, Ann Appl Biol, 123:193-219). During senescence some of these variants retain both chlorophyll and photosynthetic competence ("functional" stay-greens). Others keep their chlorophyll but degrade some other components of the photosynthetic apparatus as normal so they cannot utilize the light energy they have harvested ("non-functional" stay greens). Thus measurement

of greenness in general is not always sufficient to determine the progression of leaf senescence. Healthy stay-green plants produce a higher yield. Such plants may also have increased resistance to disease and drought and possess leaves with higher nutritional quality and attractiveness to grazing animals. Their retention of chlorophyll makes then an ideal source of this pigment for the food industry and ensures that amenity and ornamental plants remain attractive over an extended period.

Although these naturally-occuring genetic variants with contrasting senescence phenotypes exist (differing in the timing and rate of senescence), the use of such variants in the breeding of improved crop varieties is not entirely satisfactory. For example, research has shown that superficially similar stay-greens differ greatly in metabolism and genetic make-up. It is insufficient simply to select stay-greens by eye: potential disadvantages as well as benefits must be considered in crop management and breeding programmes. Normally, loss of green coloration (chlorophyll) directly reflects cessation of photosynthesis. In some stay-greens both processes are simply delayed; in others they occur at the normal time, but more slowly than usual. But in others, chlorophyll is retained despite loss of photosynthetic activity (although leaves retain more protein and lipid). Thus the stay-green character in one genetic line may have only a superficial resemblance to the character in another and may arise from quite different underlying physiological and biochemical modifications.

It is an object of the present invention to

provide a means to modify senescence at the level of gene expression.

The modification of plant gene expression has been achieved by several methods. The molecular biologist can choose from a range of known methods to decrease or increase gene expression or to alter the spatial or temporal expression of a particular gene. For example, the expression of either specific antisense RNA or partial sense RNA has been utilised to reduce the expression of various target genes in plants (as reviewed by Bird and Ray, 1991, Biotechnology and Genetic Engineering Reviews 9:207-227). These techniques involve the incorporation into the genome of the plant of a synthetic gene designed to express either antisense or sense RNA. They have been successfully used to down-regulate the expression of a range of individual genes, for example those involved in the development and ripening of fruit (Gray et al, 1992, Plant Molecular Biology, 19:69-87). Methods to increase the expression of a target gene have also been developed. For example, additional genes designed to express RNA containing the complete coding region of the target gene may be incorporated into the genome of the plant to "over-express" the gene product. Various other methods to modify gene expression are known; for example, the use of alternative regulatory sequences.

Senescence-related genes having a function in foliar senescence may be classified according to their patterns of expression during leaf development (Smart, 1994, New Phytol, 126:419-448; Thomas, 1994, Reviews in Clinical Gerontology,

4:5-20). Six broad categories may be recognised:

(1) "Housekeeping" genes controlling the primary metabolic activities of viable cells(such as respiration, ribosomal RNA and protein synthesis);

(2) Genes expressed early, whose effects become apparent later (for example, homeotic genes and genes encoding mRNAs or proteins such as vacuolar enzymes or zymogens that become active later in the life of the leaf);

(3) Genes which encode growth or carbon assimilation components and contribute to the progress of senescence by switching off (such as nuclear and plastid genes for Calvin cycle enzymes and thylakoid proteins);

(4) Regulatory genes that are expressed at the initiation of senescence and control its timing and rate of progress;

(5) Genes encoding RNAs or proteins induced de novo or showing enhanced expression during senescence (such as enzymes of pigment breakdown);

(6) Genes encoding proteins which remobilise storage products (such as enzymes of gluconeogenesis).

According to the present invention there is provided a method of inhibiting or accelerating plant senescence by modifying the expression of at least one senescence-related gene.

A senescence-related gene is a gene which has a role in senescence. The senescence-related gene may be activated during senescence, or may be down-regulated during senescence, or may show an unchanged level of expression during senescence.

Senescence may be inhibited by inhibiting a gene which is normally activated during senescence. Additionally or alternatively, senescence may be inhibited by increasing the expression of a gene which is normally down-regulated during senescence.

Senescence may also be delayed or slowed by transforming a plant with a construct in which a promoter from a senescence-activated gene drives expression of a strong senescence antagonist.

Senescence may be accelerated by inhibiting a gene which is normally down-regulated during senescence. Additionally or alternatively, senescence may be accelerated by increasing the expression of a gene which is normally activated during senescence.

Additionally or alternatively, senescence may be inhibited or accelerated by respectively inhibiting or increasing the expression of a gene which normally shows an unchanged level of expression during senescence.

The method of the present invention can be applied to any plant, including tomato, lettuce, broccoli, cabbage, carrot, beet, melon, banana, strawberry, wheat, maize rice, canola, rape, sunflower, soybean. Selected plants will show modified senescence phenotypes, which may include one or more of the following characteristics:

(1) Prolonged life of the plant or plant part due to delayed or slowed senescence.
(2) Increased yield due to delayed senescence of the leaf and resulting prolonged

photosynthetic activity.

(3) Increased protein content of fruits and vegetables due to reduced rate of protein breakdown.

(4) Improved quality of leafy vegetables due to the reduced rate of senescence.

(5) Improved disease tolerance due to the presence of less senescing tissue.

(6) Improved tolerance of drought and other stresses.

(7) Improved storage life of the harvested plant or plant part.

(8) Highly-adapted crop in which the senescence process is under external control: onset and rate of senescence can be adapted to the specific environment or other requirements by producing crops with particular characteristics or by inducing those characteristics when needed.

(9) Increased senescence breaking down unwanted plant material more rapidly (for example, avoiding the use of desiccants on crops such as rape).

For example, delaying senescence in grain maize, sorghum, wheat or barley can have various beneficial effects on crop phenotype. The resulting increase in leaf area (increased duration of green leaf area) and rise in photosynthetic capacity during grain-filling results in increased yield. Improved stalk integrity (due to delayed senescence in stalk tissue) increases resistance to pathogenic organisms and allows improved harvesting; yield is again increased. Delaying senescence in early-maturing silage maize or sorghum will maintain leaf integrity and greenness

throughout the continuing growing season to increase the crop's overall biomass.

Naturally-occurring stay-green variants can be used as parents in crop breeding programmes. However, the existence of visually identical but physiologically different "functional" and "non-functional" stay-greens complicates their use and the desired effect on crop phenotype is not easily achieved. Also it is uncertain how the stay-green character will behave when crossed into a different genetic background. In contrast, the genetic modification of single genes is a simpler process allowing a higher degree of control of genotype and phenotype. The present invention provides a means of transferring the "modified senescence" trait into elite lines without a prolonged breeding programme which might alter other beneficial traits at the same time. In addition, the generation of improved crop varieties is not limited by the naturally-available genotypes: it is possible to generate a different range of allelic forms (for example, those having greatly inhibited or greatly accelerated senescence). Genetic modification of single genes will probably result in a dominant "modified senescence" phenotype, which can be more easily incorporated into traditional breeding programmes.

In one embodiment of the invention, stay-green plant lines could be produced by inhibiting the chlorophyll degradation pathway or by preventing lipid degradation. For example, stay-green silage maize could be produced with improved quality and palatability and with post-harvest stability. The putative pathway of chlorophyll breakdown in

senescence involves various enzymes including chlorophyllase, magnesium dechelatase, dioxygenase, proteases and ferredoxin (Matile, 1992). The putative pathway of galactolipid breakdown in senescence involves gluconeogenesis and hence the enzyme pyruvate, orthophosphate dikinase which converts pyruvate to PEP (Matile, 1992).

In another embodiment, low proteolysis plant lines could be produced by down-regulating physiological protein mobilisation or by preventing autolytic degradation. For example, low proteolysis maize lines would have high silage quality and allow improved nitrogen intake and retention by the animal, with a consequent reduction in slurry nitrogen.

Genetic modification of single senescence-related genes may also be used in combination with a "gene switch" allowing control of the senescence process according to circumstances. If the relevant senescence-related genes could be switched on or off at will, the timing and progress of senescence and the phenotype of the crop could be directly controlled. For example, induction of senescence ("switching on" senescence) can allow early harvest of the crop. Modifying the expression of senescence-related genes using a gene switch may also be used to improve the quality of the crop. For example, induction of senescence in late-maturing silage maize or sorghum can result in better quality silage and better harvest timing. Silage consists of about 50% grain and 50% leaf/stem material. For late-maturing silage varieties, there is little dry matter accumulation at the end of the growing

season as the weather becomes colder. However, the moisture content increases while the crop is left in the field which reduces the quality of the silage. The ability to induce senescence at the time when maximum dry matter has been accumulated can reduce the final moisture content by around 35%, concentrating the carbohydrate material and improving silage quality.

According to a further aspect of the present invention, there is provided a method for producing plants having modified senescence characteristics which comprises transformation of plants with a DNA construct adapted to modify the expression of at least one senescence-related gene and subsequent selection of plants in which the senescence process is either inhibited or accelerated.

The expression of the or each senescence-related gene may be either reduced or increased depending on the characteristics desired for the modified plant. "Antisense" or "partial sense" or other techniques may be used to reduce gene expression or expression may be increased, for example, by incorporation of additional senescence-related genes. The additional genes may be designed to give either the same or different spatial and temporal patterns of expression in the plant.

The invention further provides a DNA construct adapted to modify the expression of at least one senescence-related gene comprising a DNA sequence corresponding to at least part of a senescence-related gene preceded by a transcriptional initiation region operative in

plants so that the construct can generate RNA in plant cells.

A DNA construct according to the invention may be an "antisense" construct generating "antisense" RNA or a "sense" construct (encoding at least part of the functional gene product) generating "sense" RNA. "Antisense RNA" is an RNA sequence which is complementary to a sequence of bases in the corresponding mRNA: complementary in the sense that each base (or the majority of bases) in the antisense sequence (read in the 3′ to 5′ sense) is capable of pairing with the corresponding base (G with C, A with U) in the mRNA sequence read in the 5′ to 3′ sense. Such antisense RNA may be produced in the cell by transformation with an appropriate DNA construct arranged to generate a transcript with at least part of its sequence complementary to at least part of the coding strand of the relevant gene (or of a DNA sequence showing substantial homology therewith). "Sense RNA" is an RNA sequence which is substantially homologous to at least part of the corresponding mRNA sequence. Such sense RNA may be produced in the cell by transformation with an appropriate DNA construct arranged in the normal orientation so as to generate a transcript with a sequence identical to at least part of the coding strand of the relevant gene (or of a DNA sequence showing substantial homology therewith). Suitable sense constructs may be used to inhibit gene expression (as described in International Patent Publication WO91/08299) or to over-express the enzyme.

The constructs of the invention may be inserted into any plant to regulate the expression

of one or more senescence-related genes. The constructs may be transformed into any dicotyledonous or monocotyledonous plant. Depending on the nature of the construct, expression of the senescence-related gene may be increased or reduced, either throughout or at particular stages in the life of the plant. Generally, as would be expected, gene expression is enhanced only by full-length sense constructs which express RNA homologous to the substantially complete coding region of the gene. Constructs containing an incomplete DNA sequence shorter than that corresponding to the complete gene generally inhibit the expression of the gene, whether they are arranged to express sense or antisense RNA. Full-length antisense constructs also inhibit gene expression.

In a DNA construct according to the invention, the transcriptional initiation region may be derived from any plant-operative promoter. The transcriptional initiation region may be positioned for transcription of a DNA sequence encoding RNA which is complementary to a substantial run of bases in a senescence-related mRNA (making the DNA construct a full or partial antisense construct).

DNA constructs according to the invention may comprise a DNA sequence at least 10 bases (preferably at least 20 bases) in length for transcription into sense or antisense RNA. There is no theoretical upper limit to the base sequence - it may be as long as the relevant mRNA produced by the cell - but for convenience it will generally be found suitable to use sequences between 100 and 1000 bases in length. The preparation of such

constructs is described in more detail below.

The sequence of any senescence-related gene may be used in the DNA constructs as suitable genes may be isolated from any plant species. For example, in work leading to this invention we have identified a range of senescence-related genes. The cDNA of these genes has been cloned and characterised, and may be used to modify the senescence process of plants, including foliar senescence, fruit senescence and senescence of other plant parts. The genes in question are encoded in the following clones:
pSENU1, pSENU2, pSENU3, pSENU4, pSENU5, pSEND31, pSEND32, pSEND33, pSEND34, pSEND35, pSENE71 (all isolated from tomato: the pSENU clones correspond to genes which are up-regulated during senescence; the pSEND clones correspond to genes which are down-regulated during senescence; the pSENE clone corresponds to a gene which exhibits even expression during senescence);
SEE1, SEE2, SEE3, SEE4, SEE5, SEE6, SEE7, SEE8, SEE9, SEC1, SEC2, SED1, SED2 (all isolated from maize; the SEE clones correspond to genes whose expression is enhanced during senescence; the SED clones correspond to genes whose expression is diminished during senescence; the SEC clones correspond to genes whose expression is constant during senescence).

The DNA sequence in a construct according to the invention may be derived from cDNA, genomic DNA or synthesised ab initio.

It is convenient to obtain or derive the DNA sequence from the sequence of any one of the cDNA

clones pSENU1, pSENU2, pSENU3, pSENU4, pSENU5, pSEND31, pSEND32, pSEND33, pSEND34, pSEND35, pSENE71, SEE1, SEE2, SEE3, SEE4, SEE5, SEE6, SEE7, SEE8, SEE9, SEC1, SEC2, SED1, SED2. Full and partial base sequences of some of these clones are set out in SEQ ID NOs 1 to 39. The following clones were deposited at The National Collections of Industrial and Marine Bacteria (23 St Machar Drive, Aberdeen, Scotland, AB2 1RY) under the terms of the Budapest Treaty on 13 July 1993 under the following accession numbers:

pSENU1	NCIMB 40571
pSENU2	NCIMB 40572
pSENU3	NCIMB 40573
pSENU4	NCIMB 40574
pSENU5	NCIMB 40575
pSEND31	NCIMB 40576
pSEND32	NCIMB 40577
pSEND33	NCIMB 40578
pSEND34	NCIMB 40579
pSEND35	NCIMB 40580
pSENE71	NCIMB 40581
SEE1	NCIMB 40582
SEE2	NCIMB 40584
SEE3	NCIMB 40570
SEE4	NCIMB 40583

cDNA clones similar to pSENU1-5 or pSEND31-35 or pSENE71 may be obtained from the mRNA of senescent tomatoes leaves. cDNA clones similar to SEE1-4 may be obtained from the mRNA of senescent maize leaves. In this way may be obtained sequences coding for the whole, or substantially the whole, of the mRNA produced by the cDNA clones.

An alternative source of DNA for the base sequence for transcription is a suitable genomic

sequence encoding a senescence-related mRNA. This gene may differ from the cDNA in that introns may be present. The introns are not transcribed into mRNA (or, if so transcribed, are subsequently cut out). When using such a gene as the source of the base sequence for transcription it is possible to use either intron or exon regions - both are useful in modifying the expression of the gene according to the invention. Oligonucleotide probes or the cDNA clone may be used to isolate the actual senescence-related gene(s) by screening genomic DNA libraries. Such genomic clones may include control sequences operating in the plant genome. Thus it is also possible to isolate promoter sequences which may be used to drive expression of the senescence-related protein or any other protein. These promoters may be particularly responsive to senescence-related events and conditions. Senescence-related promoters may be used to drive expression of any target gene.

A further way of obtaining a suitable DNA base sequence for transcription is to synthesise it ab initio from the appropriate bases, for example using the sequences in SEQ ID NOs 1 to 39The proposed full sequence of SENU2 by comparison with clone C14 is shown as SEQ ID NO 5 as a guide.

Recombinant DNA constructs according to the present invention may be made using standard techniques. For example, the DNA sequence for transcription may be obtained by treating a vector containing said sequence with restriction enzymes to cut out the appropriate segment. The DNA sequence for transcription may also be generated by annealing and ligating synthetic oligonucleotides

or by using synthetic oligonucleotides in a polymerase chain reaction (PCR) to give suitable restriction sites at each end. The DNA sequence is then cloned into a vector containing upstream promoter and downstream terminator sequences. If antisense DNA is required, the cloning is carried out so that the cut DNA sequence is inverted with respect to its orientation in the strand from which it was cut.

In a construct expressing antisense RNA, the strand that was formerly the template strand becomes the coding strand, and vice versa. The construct will thus encode RNA in a base sequence which is complementary to some or all of the sequence of the senescence-related mRNA. Thus the two RNA strands are complementary not only in their base sequence but also in their orientations (5' to 3').

In a construct expressing sense RNA, the template and coding strands retain the assignments and orientations of the original plant gene. Constructs expressing sense RNA encode RNA with a base sequence which is homologous to part or all of the sequence of the mRNA. In constructs which express the functional senescence-related gene product, the whole of the coding region of the gene is linked to transcriptional control sequences capable of expression in plants.

For example, constructs according to the present invention may be made as follows. A suitable vector containing the desired base sequence for transcription (such as pSEND35) is treated with restriction enzymes to cut the

sequence out. The DNA strand so obtained is cloned (if desired, in reverse orientation) into a second vector containing the desired promoter sequence (for example cauliflower mosaic virus 35S RNA promoter or the promoter of the pSENU1 gene or other genes which are switched on at the onset of senescence) and the desired terminator sequence (for example the 3' of the Agrobacterium tumefaciens nopaline synthase gene, the nos 3' end).

The transcriptional initiation region (or promoter) operative in plants may be a constitutive promoter (such as the 35S cauliflower mosaic virus promoter) or an inducible or developmentally regulated promoter (such as fruit-specific promoters), as circumstances require. Use of a constitutive promoter will tend to affect the senescence process in all parts of the plant, while use of a tissue specific promoter allows more selective control of gene expression and affected functions as the antisense or sense RNA is only produced in the organ in which its action is required. For example, fruit development and/or ripening-specific promoters that could be used include the ripening-enhanced polygalacturonase promoter (International Patent Publication Number WO92/08798), the E8 promoter (Diekman & Fischer, 1988, EMBO, 7:3315-3320) and the fruit specific 2A11 promoter (Pear et al, 1989, Plant Molecular Biology, 13:639-651). Inducible promoters may also be used. According to a further aspect of the invention, the transcriptional initiation region in the DNA construct comprises a "gene switch".

Several gene promoter sequences are known

which are responsive to an applied exogenous chemical inducer. This enables external control of expression of the gene controlled by the inducible promoter. For example, European patent application publication number EPA 332104 (published 18th September 1989) describes chemically regulatable DNA sequences isolated from the pathogenesis-related (PR) protein gene; International patent application publication numbers WO90/08826 (published 9 August 1990) and WO93/01294 (published 21 January 1993) describe a chemically inducible gene promoter sequence isolated from a 27kd subunit of the maize glutathione-S-transferase gene (GST II-27); International patent application number GB93/00764 describes a chemically-inducible gene expression cassette including a regulator protein (such as the Aspergillus nidulans alcR protein) and an inducible promoter (such as the A nidulans alcA promoter).

Such chemically-inducible promoter sequences may be used in "gene switches" to regulate transcription of an associated DNA sequence (or "target gene") in plants or plant tissue.

The gene switch may be a positive switch, where the inducible promoter directly controls the target gene. In the presence of the chemical inducer, the target gene is switched on and the encoded protein is expressed.

For example, the inducible GST II-27 promoter can be operatively linked to one or more target genes to give a chemically switchable construct: expression of the target gene(s) is controlled by application of an effective exogenous inducer. The

gene switch construct may be inserted into a plant by transformation. The inducible GST II-27 promoter is functional in both monocotyledons and dicotyledons, and in a variety of tissues including roots, leaves, stems and reproductive tissues. Effective inducers for use with the GST II-27 promoter include N,N-diallyl-2,2-dichloroacetamide (common name: dichloramid); benzyl-2-chloro-4-(trifluoromethyl) -5-thiazole-carboxylate (common name: flurazole); naphthalene-1,8-dicarboxylic anhydride; 2-dichloromethyl-2-methyl-1,3-dioxolane and several others as described in International patent application publication numbers WO90/08826 and WO93/01294. The contents of the said applications are incorporated herein by reference.

Alternatively, the gene switch may be a negative switch, where the inducible promoter indirectly controls the target gene via a repressor/operator system. In the presence of the chemical inducer, the target gene is switched off and the encoded protein is not expressed.

For example, negative gene switches are described in International patent application publication numbers WO90/08829, WO90/08827 and WO90/08830 (all published 9 August 1990). The contents of the said applications are incorporated herein by reference. The switch comprises a chemically-inducible promoter (A) driving expression of a repressor gene encoding a repressor protein, and a promoter (B) containing an operator sequence and driving expression of a target gene. (The operator region may be introduced into promoter(B) by biotechnological techniques). If

present, the repressor protein binds to the operator sequence, preventing expression of the target gene. In the absence of inducer, promoter (A) is not active and the repressor protein is not expressed: hence the target gene is expressed. In the presence of the chemical inducer, the repressor protein prevents expression of the target gene. Promoter (A) may be GST II-27 or any other chemically-inducible promoter sequence. The repressor gene/operator sequences may be taken from the E coli lac operon.

Senescence-related gene expression (and hence senescence characteristics) may be modified to a greater or lesser extent by controlling the degree of sense or antisense mRNA production in the plant cells. This may be done by suitable choice of promoter sequences, or by selecting the number of copies or the site of integration of the DNA sequences that are introduced into the plant genome. For example, the DNA construct may include more than one DNA sequence encoding a senescence-related gene or more than one recombinant construct may be transformed into each plant cell.

It is also possible to modify the activity of the senescence-related gene while also modifying the activity of one or more other plant genes. For example, a first plant may be individually transformed with a senescence-related DNA construct and then crossed with a second plant which has been individually transformed with a construct adapted to modify the expression of another gene. As a further example, single plants may be either consecutively or co-transformed with

senescence-related DNA constructs and with appropriate constructs for modification of the other gene(s). An alternative example is plant transformation with a senescence-related DNA construct which itself contains an additional gene for modification of the activity of the other gene(s). The senescence-related DNA constructs may contain sequences of DNA for regulation of the expression of the other gene(s) located adjacent to the senescence-related sequences. These additional sequences may be in either sense or antisense orientation as described in International patent application publication number WO93/23551 (single construct having distinct DNA regions homologous to different target genes). By using such methods, the benefits of modifying the activity of the senescence-related gene may be combined with the benefits of modifying the activity of other genes.

The senescence characteristics of plants may be modified by transformation with a DNA construct according to the invention. The invention further provides plant cells containing constructs of the invention; plants derived therefrom showing modified senescence characteristics; and seeds of such plants.

A DNA construct of the invention is transformed into a target plant cell. The target plant cell may be part of a whole plant or may be an isolated cell or part of a tissue which may be regenerated into a whole plant. The target plant cell may be selected from any monocotyledonous or dicotyledonous plant species. Suitable plants include any fruit-bearing plant (such as tomatoes, mangoes, peaches, apples, pears, strawberries,

bananas and melons) and other important crops such as maize, rice, wheat, barley, sorghum, sugar beet, canola, rape, soybean. For any particular plant cell, the DNA sequence used in the transformation construct may be derived from the same plant species, or may be derived from any other plant species (as there will be sufficient sequence similarity to allow modification of related enzyme gene expression).

Constructs according to the invention may be used to transform any plant using any suitable transformation technique to make plants according to the invention. Both monocotyledonous and dicotyledonous plant cells may be transformed in various ways known to the art. In many cases such plant cells (particularly when they are cells of dicotyledonous plants) may be cultured to regenerate whole plants which subsequently reproduce to give successive generations of genetically modified plants. Any suitable method of plant transformation may be used. For example, dicotyledonous plants such as tomato and melon may be transformed by Agrobacterium Ti plasmid technology, such as described by Bevan (1984, Nucleic Acid Research, 12:8711-8721) or Fillatti et al (Biotechnology, July 1987, 5:726-730). Such transformed plants may be reproduced sexually, or by cell or tissue culture.

The invention will now be described by way of example only, with reference to the SEQUENCE LISTING and to the drawings in which:

Figure 1 is a diagram illustrating the construction of a pSENU1 sense construct.

Figure 2 is a diagram illustrating the

construction of a pSENU1 antisense construct.

Figure 3 is a diagram illustrating the construction of a pSENU5 sense construct.

Figure 4 is a diagram illustrating the construction of a pSENU5 antisense construct.

EXAMPLE 1

Characterisation of tomato senescence-related clones

RNA was extracted from tomato plants at four stages of senescence: mature green (no yellowing), onset (first visible sign of colour loss), mid (about 30% colour loss) and advanced (completely yellow). A tomato leaf senescence cDNA library was constructed using polyA$^+$ RNA from the onset and mid stages of senescence in the ration 4:1. A Stratagene lambda Uni-ZAP XR cDNA library was generated with 1x10^6 pfu, average insert size 0.9kB. Amplification gave 500,000 clones which were differentially screened (Hodge et al, 1992, Plant Journal, 2:257-260) using mature green and onset/mid polyA$^+$ single-stranded cDNA. Genes differentially expressed during leaf senescence in tomato were isolated and characterised. Details of the cDNA clones are given below.

(1) <u>pSENU1</u>

pSENU1 (also known as clone 4S1) is a cDNA of approximately 1.0kB, encoding a mRNA of approximately 1.4 kB. The mRNA encoded by pSENU1 is expressed during the onset of senescence in tomato leaves.

pSENU1 encodes a protein of unknown function. The pSENU1 DNA sequence does not show any significant homology to sequences in publicly-available sequence databases.

The sequence of pSENU1 is shown as SEQ ID NO 1. The clone was deposited at The National Collections of Industrial and Marine Bacteria (Scotland) on 13 July 1993 under the accession number NCIMB 40571.

(2) pSENU2

pSENU2 (also known as clone 8S1) is a cDNA of approximately 1.2kB, encoding a mRNA of approximately 1.4 kB. The mRNA encoded by pSENU2 is expressed during the onset of senescence in tomato leaves.

The pSENU2 sequence exhibits 100% homology with C14 cDNA induced in unripe tomato fruit in response to low temperature. DNA sequence analysis indicated that C14 mRNA encodes a polypeptide with a region that is homologous to the plant thiol proteases actinidin and papain and to animal thiol protease cathepsin H (Schaffer and Fischer, 1988, Plant Physiol, 87:431-436). A similar thiol protease gene is also expressed in pea ovaries during senescence (Granell et al, 1992, Plant J, 2:907-915). We have now found that this kind of protease is also expressed during leaf senescence. This protease may play an important role in the degradation of peptides at the onset and during foliar senescence.

Partial sequences of pSENU2 are shown as SEQ

ID NO 2 (5' end) to SEQ ID NO 4 (3' end). The proposed full sequence of SENU2 by comparison with clone C14 is shown as SEQ ID NO 5. The clone pSENU2 was deposited at The National Collections of Industrial and Marine Bacteria (Scotland) on 13 July 1993 under the accession number NCIMB 40572.

(3) pSENU3

pSENU3 (also known as clone 77S3) is a cDNA of 1.1982kB, encoding a mRNA of approximately 1.4 kB. The mRNA encoded by pSENU3 is expressed during the onset of senescence in tomato leaves.

The pSENU3 sequence exhibits 70% homology with oryzain gamma, a cysteine proteinase expressed in rice seeds and induced by gibberellin, GA3 (Watanabe et al, 1991, J Biol Chem, 266:16897-16902). We have now identified a related gene (pSENU3) expressed in leaves.

The complete sequence of pSENU3 is shown as SEQ ID NO 6. The clone was deposited at The National Collections of Industrial and Marine Bacteria (Scotland) on 13 July 1993 under the accession number NCIMB 40573.

(4) pSENU4

pSENU4 (also known as clone 73S7) is a cDNA of 0.525kB, encoding a mRNA of approximately 0.7kB. The clone is double-stranded from base 179 to base 323; the rest is single-stranded. The mRNA encoded by pSENU4 is expressed during the onset of senescence in tomato leaves.

The predicted amino acid sequence encoded by pSENU4 matches that originally determined by Lucas et al (1985, EMBO J, 4:2745-2749) for the tomato extracellular pathogenesis related protein P14. The P14 protein (PR14_LYCES) contains two isomers, P4 and P6 (van Kan et al, 1992, Plant Mol Biol, 20:513-527). P6 is 15.5kD and is serologically related to the PR1 protein family of tobacco, but has not been assigned a function. The pSENU4 sequence is homologous to the cDNA encoding P6 (clone LEPRP6) which has been isolated from Cladosporium fulvum infected tomato, but pSENU4 lacks 160bp at the 5'end of the P6 cDNA. An identical cDNA (clone LEP1P14A) has also been isolated from ethylene treated tissue (Vera P and Tonero P, unpublised data) which encodes a protein named P1(P14a).

The complete sequence of pSENU4 is shown as SEQ ID NO 7. The clone was deposited at The National Collections of Industrial and Marine Bacteria (Scotland) on 13 July 1993 under the accession number NCIMB 40574.

(5) pSENU5

pSENU5 (also known as clone 72S3) is a cDNA of 0.847kB, encoding a mRNA of approximately 2.0kB. The mRNA encoded by pSENU5 is expressed during the onset of senescence in tomato leaves.

pSENU5 encodes a protein of unknown function. The pSENU5 DNA sequence does not show any significant homology to sequences in publicly-available sequence databases.

The complete sequence of pSENU5 is shown as SEQ ID NO 8. The clone was deposited at The National Collections of Industrial and Marine Bacteria (Scotland) on 13 July 1993 under the accession number NCIMB 40575.

(6) pSEND31

pSEND31 (also known as clone 1M4) is a cDNA of approximately 0.9kB, encoding a mRNA of approximately 1.0kB. The mRNA encoded by pSEND31 is expressed in green leaves of tomatoes plants but at the onset of senescence its expression is switched off.

The pSEND31 sequence exhibits 100% homology to the tomato cDNA clone TAS14 which is inducible by salt stress and ABA in tomato seedlings (Godoy et al, 1990, Plant Mol Biol, 15:695-705). Southern analysis suggests that there is one gene per haploid genome. We now show this gene is specifically reduced during tomato leaf senescence.

Partial sequences of pSEND31 are shown as SEQ ID NO 9 (5' end) to SEQ ID NO 10 (towards the 3' end). The proposed full sequence of SEND31 by comparison with clone TAS14 is shown as SEQ ID NO 11. The clone pSEND31 was deposited at The National Collections of Industrial and Marine Bacteria (Scotland) on 13 July 1993 under the accession number NCIMB 40576.

(7) pSEND32

pSEND32 (also known as clone 8M2) is a cDNA of 0.8kB, encoding a mRNA of approximately 0.6kB. The

mRNA encoded by pSEND32 is expressed in green leaves of tomatoes plants but at the onset of senescence its expression is switched off.

pSEND32 encodes a protein of unknown function. The pSEND32 DNA sequence does not show any significant homology to sequences in publicly-available sequence databases.

The sequence of pSEND32 is shown as SEQ ID NO 12. The clone was deposited at The National Collections of Industrial and Marine Bacteria (Scotland) on 13 July 1993 under the accession number NCIMB 40577.

(8) pSEND33

pSEND33 (also known as clone 59S3) is a cDNA of approximately 0.8kB, encoding a mRNA of approximately 0.6kB. The mRNA encoded by pSEND33 is expressed in green leaves of tomatoes plants but at the onset of senescence its expression is switched off.

The pSEND33 sequence exhibits 60% homology to ferredoxin-1 of pea and spinach. The amino acid sequence of plant ferredoxins is highly conserved. Plant leaves contain at least two distinct forms of chloroplast-type ferredoxins. Ferredoxin-1 appears more closely related to other angiosperm ferredoxins. Ferredoxin-1 is encoded by a single gene in pea (Elliott et al, 1989, Plant Cell, 1:681-690).

pSEND33 may encode ferredoxin. We have now shown that expression of this gene is specifically

reduced during tomato leaf senescence. The down-regulation of ferredoxin during senescence may be counteracted by overexpression of the pSEND33 gene. This may lead to prolonged photosynthesis and increased yield of plants.

The sequence of pSEND33 is shown as SEQ ID NO 13. The clone was deposited at The National Collections of Industrial and Marine Bacteria (Scotland) on 13 July 1993 under the accession number NCIMB 40578.

(9) pSEND34

pSEND34 (also known as clone 91S3) is a cDNA of 0.558kB, encoding a mRNA of approximately 0.6kB. The mRNA encoded by pSEND34 is expressed in green leaves of tomatoes plants but at the onset of senescence its expression is switched off.

The pSEND34 sequence exhibits 90% homology to the potato photosystem II 10kD polypeptide at both the nucleotide and the predicted amino acid level (Eckes et al, 1986, Mol Gen Genet, 205:14-22). Homology with the same protein from spinach and Arabidopsis is about 80% (Lautner et al, 1988, J Biol Chem, 263:10077-10081; Gil-Gomez et al, 1991, Plant Mol Biol, 17:517-522). We have now shown that this gene is specifically reduced during tomato leaf senescence.

The sequence of pSEND34 is shown as SEQ ID NO 14. The clone was deposited at The National Collections of Industrial and Marine Bacteria (Scotland) on 13 July 1993 under the accession number NCIMB 40579.

(10) pSEND35

pSEND35 (also known as clone 72S6) is a cDNA of approximately 0.7kB. The mRNA encoded by pSEND35 is expressed in green leaves of tomatoes plants but at the onset of senescence its expression is switched off.

pSEND35 encodes a protein of unknown function. The pSEND35 DNA sequence does not show any significant homology to sequences in publicly-available sequence databases.

The sequence of pSEND35 is shown as SEQ ID NO 15. The clone was deposited at The National Collections of Industrial and Marine Bacteria (Scotland) on 13 July 1993 under the accession number NCIMB 40580.

(11) pSENE71

pSENE71 (also known as clone 75S3) is a cDNA of 0.6kB. The mRNA encoded by pSENE71 is expressed in green leaves of tomatoes plants and during senescence.

pSENE71 encodes a protein of unknown function. The pSENE71 DNA sequence does not show any significant homology to sequences in publicly-available sequence databases.

The 5′ sequence of pSENE71 is shown as SEQ ID NO 16. The clone was deposited at The National Collections of Industrial and Marine Bacteria (Scotland) on 13 July 1993 under the accession

number NCIMB 40581.

EXAMPLE 2

Characterisation of maize senescence-related clones

(A) SUMMARY

Leaf senescence after pollen shed was studied in two maize lines. Relative chlorophyll content, photosystem II efficiency (determined by analysis of chlorophyll fluorescence) and photosynthetic CO_2 fixation (measured by infrared gas analyser) declined during senescence. Statistical analysis of the fitted curves revealed that yellowing in the first line was significantly delayed compared with the second line, but the decline in photosynthesis occurred simultaneously in the two lines. Western blotting detected a transition point during senescence when pronounced quantitative and qualitative changes occurred in a number of leaf proteins. This point, coinciding with the onset of visible senescence, was delayed in the first line. Changes in the complement of translatable mRNAs were apparent earlier than alterations in pigment or protein levels. A cDNA library was constructed from the poly(A)$^+$ RNA of leaves judged to be in the early stages of senescence and differential screening was employed to isolate senescence-related clones, which were investigated further by northern analysis. Partial sequencing, followed by comparison with all known sequences in the GenEMBL database, indicated that a number of cDNAs were related to genes of known identity, including oryzain, pyruvate, orthophosphate dikinase and ferredoxin I, while others showed similarity to

cDNAs of unknown function, or did not exhibit any significant homology.

(B) EXPERIMENTAL DETAILS

Non-destructive measurements of chlorophyll fluorescence and the level of greenness were made on ear leaves from two maize genotypes (X and Y). Ear leaves were harvested at intervals from 5 days before pollen shed until 35 days after pollen shed. The data indicated a significant decrease in the chlorophyll fluorescence parameter and greenness between 20 and 25 days after pollen shed (daps) for Y, and between 25 and 30 daps for X. The decrease in the photosynthetic rate with increasing leaf age is reflected in the increase in the sub-stomatal CO_2 concentration with age in both genotypes. Western analysis using proteins extracted from the same leaves gave a pattern of bands obtained with different antibodies which confirmed that a change occurs between 20 and 25 daps for Y and between 25 and 30 daps for X. Preliminary results of cell-free translation using total RNA extracted from the same leaves suggested that subtle increases in the intensity of certain bands are detectable 10 days after pollen shed, although more obvious changes are not seen until 20-25 days after pollen shed.

The evidence from the physiological and molecular data suggested that protein and RNA changes occur before visible senescence takes place. Two cDNA libraries were therefore made: one from RNA extracted from leaves 10-20 days after pollen shed (initiation/early senescence) and the other from RNA extracted from leaves 25 days after pollen shed (mid senescence).

Total RNA was extracted from leaves at the appropriate stages, polyA$^+$ RNA was purified and cDNA made using a Pharmacia cDNA Synthesis Kit. Two cDNA libraries (early and mid senescence) were constructed in lambda gt10 (average insert size 1.3 kB). Senescence-related cDNA clones were identified by differential screening, using RNA from leaves at pollen shed and RNA from the same stages as were selected to make the library to provide the necessary probes.

Clones showing stronger hybridisation with a senescing leaf cDNA probe than with a mature green leaf cDNA probe were further characterised. Both lambda minipreps and PCR were utilised to isolate and determine the size of cDNA inserts. Duplicate cDNA clones were identified by Southern analysis. A number of independent cDNA clones were used as probes on Northern analysis to investigate the level of mRNA in different ages of leaves. The level of mRNA increases with leaf age in genotype Y but not in X for a number of cDNA clones (including SEE4). This suggests that these messages become more abundant in normal senescence but that their expression is blocked in some way in the stay-green genotype X. Other messages, such as those hybridising to the cDNAs SEE1 and SEE2, increase with leaf age in both genotypes. These mRNAs may form part of the senescence syndrome which occurs on schedule in both genotypes.

(C) SENESCENCE-RELATED GENES

Details of some of the cDNAs (including their approximate sizes and sizes of the corresponding mRNAs) are given below. End-sequencing of the

cDNAs allowed comparison with known sequences in the GenEMBL database. Some homologies were found.

The cDNA SEE1 shows homology to genes for two thiol proteases, oryzain gamma from rice and aleurain from barley. The cDNA SEE2 shows some homology to a castor bean vacuolar processing enzyme. The cDNA SEE3 is identical to part of the maize pyruvate, orthophosphate dikinase mRNA while the cDNA SEE4 shows homology to maize and Silene ferredoxin mRNAs.

Proteases and ferredoxin are thought to play a part in chlorophyll breakdown during senescence, while pyruvate, orthophosphate dikinase has a role in gluconeogenesis, which has been suggested to occur during galactolipid breakdown in senescence. Hence the maize cDNAs represent senescence-related genes and may be used to modify the senescence process.

(1) SEE1 (also known as clone p16.4)

SEE1 is a senescence-enhanced cDNA clone of approximately 1.7kB, encoding a mRNA of approximately 1.2kB. The mRNA encoded by SEE1 increases in abundance during maize leaf senescence.

SEE1 shows homology to genes for two thiol proteases: oryzain gamma from rice (GenEMBL ID code OSOZC; 77.6% identity in a 304bp overlap) and aleurain from barley (GenEMBL ID code HVLEU; 77.9% identity in a 222bp overlap). A more detailed comparison of the SEE1 sequence with the rice oryzain gamma DNA sequence (Watanabe et al, 1991)

shows a 78.9% identity over 1417 base pairs, with 80% homology over 607 base pairs at the 5' end and 74% homology over 665 base pairs at the 3' end.

The clone SEE1 may thus encode a protease. This is supported by the finding that a 1kB DNA fragment is amplified by PCR when one of the primers used is derived from a region which is conserved in a range of thiol proteases.

The sequence of SEE1 is shown as SEQ ID NO 17. It is 1442 base pairs in length with a single long open reading frame between bases 78 to 1160. The predicted amino acid sequence suggests that the encoded protein is 360 amino acids long with a molecular weight of 39 kDa. There are conserved motifs in the protein sequence: a putative vacuolar signal, a Cys active site, a His active site and an Asn active site. The predominantly hydrophilic protein sequence suggests that the protein is soluble. At the protein level, the SEE1 sequence shows 85.6% identity over 355 amino acids to the thiol protease aleurain precursor and 83.7% identity over 363 amino acids to the oryzain gamma precursor.

The SEE1 clone was deposited at The National Collections of Industrial and Marine Bacteria (Scotland) on 13 July 1993 under the accession number NCIMB 40582.

(2) SEE2 (also known as clone p20.2)

SEE2 is a senescence-enhanced cDNA clone of approximately 1.3kB, encoding a mRNA of approximately 1.8kB. The mRNA encoded by SEE2

increases in abundance during maize leaf senescence.

SEE2 shows homology to a castor bean vacuolar processing enzyme (Hara-Nishimura et al, 1993): 67% homology over 374 base pairs at the 5′ end (and at the protein level, 75% homology over 130 amino acids at the 5′ end) but no homology over 431 base pairs at the 3′ end.

Partial sequences of SEE2 are shown as SEQ ID NO 18 (isolated using a T7 primer) and SEQ ID NO 19 (isolated using a T3 primer). The clone SEE2 was deposited at The National Collections of Industrial and Marine Bacteria (Scotland) on 13 July 1993 under the accession number NCIMB 40584.

(3) SEE3 (also known as clone pSE1)

SEE3 is a senescence-enhanced cDNA clone of approximately 2.5kB, encoding a mRNA of approximately 3.4kB. The mRNA encoded by SEE3 increases in abundance during maize leaf senescence.

The SEE3 sequence exhibits 100% identity to the maize pyruvate, orthophosphate dikinase mRNA (GenEMBL ID code ZMPOD) in a 141bp overlap. A more detailed comparison to the dikinase (Matsuoka et al, 1988) shows a 91% homology over 132 base pairs at the 5′ end and an 89% homology over 262 base pairs at the 3′ end. SEE3 also exhibits 97.7% identity to the maize pyruvate, orthophosphate dikinase gene, exons 2-19 (GenEMBL ID code ZMPPDK2) in a 128 bp overlap.

The sequence of SEE3 has an internal EcoRI site, and partial sequences of SEE3 are shown as SEQ ID NOs 20 to 23 (SEQ ID NO 20 being the most 5' and SEQ ID NO 23 being the 3' end). The clone SEE3 was deposited at The National Collections of Industrial and Marine Bacteria (Scotland) on 13 July 1993 under the accession number NCIMB 40570.

(4) SEE4 (also known as clone p20.0)

SEE4 is a senescence-enhanced cDNA clone of approximately 0.9kB, encoding a mRNA of approximately 1.2kB. The mRNA encoded by SEE4 increases in abundance during maize leaf senescence.

The SEE4 sequence exhibits homology to the following ferredoxin mRNAs: maize ferredoxin I isoprotein mRNA, pFD1 (GenEMBL ID code ZMFD1; 80.6% identity in a 170bp overlap); maize ferredoxin I isoprotein mRNA, pFD1' (GenEMBL ID code ZMFD1P; 80.6% identity in a 170bp overlap); maize ferredoxin isoprotein mRNA, pFD5 (GenEMBL ID code ZMFD5; 81.8% identity in a 159bp overlap); maize ferredoxin III isoprotein mRNA (GenEMBL ID code ZMFD3; 67.9% identity in a 140bp overlap); Silene pratensis mRNA for ferredoxin precursor (GenEMBL ID code SPFER1; 67.5% identity in a 120bp overlap). A more detailed comparison of the SEE4 sequence with the ferredoxin I sequence (Hase et al, 1991) shows no homology over 261 base pairs at the 5' end and 79% homology over 247 base pairs at the 3' end (with 67% homology at the protein level over 52 amino acids at the 3' end).

The 5' sequence of SEE4 is shown as SEQ ID NO

24 and the 3' sequence as SEQ ID NO 25. The clone was deposited at The National Collections of Industrial and Marine Bacteria (Scotland) on 13 July 1993 under the accession number NCIMB 40583.

(5) OTHER CLONES

SEE5 (p16.1) shows homology to a maize catalase (Abler and Scandalios, unpublished): 87% over 79 base pairs at the 3' end. The 3' sequence of SEE5 is shown as SEQ ID NO 26.

SEE6 (p16.3) shows homology to a maize cab-1 (Sullivan et al, 1989): 97% over 270 base pairs at the 5' end and 98% over 349 base pairs at the 3'end. The 5' sequence of SEE6 is shown as SEQ ID NO 27 and the 3' sequence as SEQ ID NO 28.

SEE7 (p16.5) shows homology to a maize GRP (Didierjean et al, 1992): 61% over 198 base pairs at the 5' end but no homology over 450 base pairs at the 3' end. The 5' sequence of SEE7 is shown as SEQ ID NO 28 and the 3' sequence as SEQ ID NO 30.

SEE8 (p16.6) shows no homology to known sequences over 388 base pairs at the 5' end. The partial sequence of SEE8 is shown as SEQ ID NO 31 (isolated with the T3 primer).

SEE9 (p16.11) shows no homology to known sequences over 209 base pairs at the 5' end and over 390 base pairs at the 3' end. Partial sequences of SEE9 are is shown as SEQ ID NO 32 (isolated with the T7 primer) and SEQ ID NO 33 (isolated with the T3 primer).

SEC1 (p16.7) shows homology to an Arabidopsis thaliana PS1 type III cab (Wang, Zhang and Goodman, unpublished): 79% homology over 136 base pairs at the 5′ end. The partial sequence of SEC1 is shown as SEQ ID NO 34 (isolated with the T3 primer).

SEC2 (p16.9) shows no homology to known sequences over 151 base pairs at the 5′ end and over 385 base pairs at the 3′ end. The 5′ sequence of SEC2 is shown as SEQ ID NO 35 and the 3′ sequence as SEQ ID NO 36.

SED1 (p16.13) shows homology to an Arabidopsis thaliana AP52 ATP sulfurylase (Leustek, unpublished): 62% homology over 282 base pairs at the 5′ end but no homology over 444 base pairs at the 3′ end. The 5′ sequence of SED1 is shown as SEQ ID NO 37 and the 3′ sequence as SEQ ID NO 38.

SED2 (p20.3) shows homology to an Arabidopsis thaliana cDNA 20D6T7 (Newman, unpublished): 68% over 131 base pairs at the 3′ end (and at the protein level, 85% over 26 amino acids at the 3′ end). The partial sequence of SED2 is shown as SEQ ID NO 39 (isolated with the T7 primer).

EXAMPLE 3

Construction of antisense RNA vectors with the CaMV 35S promoter.

A vector is constructed using sequences corresponding to a restriction fragment obtained from a senescence-related cDNA clone described in Example 1 or 2 and is cloned into the vectors GA643

(An et al, 1988, Plant Molecular Biology Manual A3: 1-19) or pDH51 (Pietrzak et al, 1986, Nucleic Acids Research, 14:5875-5869) which has previously been cut with a compatible restriction enzyme(s). A restriction fragment from the senescence/pDH51 clone containing the promoter, the senescence-related clone fragment and other pDH51 sequence is cloned into SLJ44026B or SLJ44024B (Jones et al, 1990, Transgenic Research, 1) or a Bin19 (Bevan, 1984, Nucleic Acids Research, 12:8711-8721) which permits the expression of the antisense RNA under control of the CaMV 35S promoter.

After synthesis of the vector, the structure and orientation of the sequences are confirmed by DNA sequence analysis.

EXAMPLE 4

Construction of antisense RNA vectors with the polygalacturonase promoter.

The fragment of the senescence-related cDNA described in Example 3 is also cloned into the vector pJR3. pJR3 is a Bin19 based vector, which permits the expression of the antisense RNA under the control of the tomato polygalacturonase promoter. This vector includes approximately 5 kb of promoter sequence and 1.8 kb of 3' sequence from the PG promoter separated by a multiple cloning site.

After synthesis, vectors with the correct orientation of senescence-related sequences are identified by DNA sequence analysis.

EXAMPLE 5

Construction of sense RNA vectors with the CaMV 35S promoter.

The fragment of senescence-related cDNA described in Example 3 is also cloned into the vectors described in Example 3 in the sense orientation.

After synthesis, the vectors with the sense orientation of senescence-related sequence are identified by DNA sequence analysis.

EXAMPLE 6

Construction of sense RNA vectors with the polygalacturonase promoter.

The fragment of senescence-related cDNA that was described in Example 3 is also cloned into the vector pJR3 in the sense orientation.

After synthesis, the vectors with the sense orientation of senescence-related sequence are identified by DNA sequence analysis.

EXAMPLE 7

Construction of an over-expression vector using the CaMV35S promoter.

The complete sequence of the senescence-related cDNA clone is inserted into the vectors described in Example 3.

EXAMPLE 8

Construction of an over-expression vector using the polygalacturonase promoter.

The complete sequence of the senescence-related cDNA clone is inserted into pJR3.

EXAMPLE 9

Generation of transformed plants

Vectors are transferred to Agrobacterium tumefaciens LBA4404 (a micro-organism widely available to plant biotechnologists) and are used to transform tomato plants.

Transformation of tomato cotyledons follows standard protocols (e.g. Bird et al Plant Molecular Biology 11, 651-662, 1988). Transformed plants are identified by their ability to grow on media containing the antibiotic kanamycin. Plants are regenerated and grown to maturity.

Plants are analysed for modifications to their senescence characteristics.

EXAMPLE 10

Construction of further vectors

Part of the senescence-related sequence encoded by SEE1 is incorporated into DNA constructs in the sense orientation and under the control of (a) the ubiquitin promoter and (b) the GST II promoter. Similar constructs are made using the SEE2 sequence. These constructs are used to transform maize.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: ZENECA LIMITED
(B) STREET: 15 Stanhope Gate
(C) CITY: London
(D) STATE: England
(E) COUNTRY: UK
(F) POSTAL CODE (ZIP): W1Y 6LN

(ii) TITLE OF INVENTION: Regulation of Senescence

(iii) NUMBER OF SEQUENCES: 39

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: GB 9318927.2
(B) FILING DATE: 13-SEP-1993

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 660 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SENU1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GTTGTGTAGC TACTACAAAC ATCATCAATA TTCAATAATG GTTCTCCAAA ATCAACTTGG 60

TCATCATCAA TCATTCTCTC ATGAACACAA CCACTATTCC GCGGAGAGCA ACCATGGACA 120

AATGATGAGG CCTTTCGCCA TGCCATTTTC TCAGTCTTCT CACAATAATC ACATGATGGT 180

CAGCCAACAC AGTGGCGGAT ATTATGGAAG CCATGGCCAT GGCCATAGCA ACTATGGAAG 240

TCATGGCCAT ATGCCACATG AATCAACCAA CTTCTCAAGT AGCACAAGCA TGGTCCATAG 300

TGATGGTGGT TATGGTAGTG GAATGAACCA ATCATCCCAC GCCCATATGT CGTCCATGGC 360

CATGGGACAT CATGGAAGTC ATGGTCATGG TCATGGACAT GGACTAGGTT TTGGTGGAAG 420

CCACCAAAAT AGCTACAGTC AGAGCCAGAA GGTCAACTGG GCTCTCAAGA ATTTGGATGA 480

CTAAATTTTA CACACACATA TATATAATAT ATGTGTGGTG AAAGTAATAT TATGTGTGTT 540

TGTGGTAGTT ACTTTGGCTA TATGTAGTAC AAAGGTTTGC TACCTAAATA AGTAAACAAT 600

CTACTGCTAT CTTAATTTAA ATTATCTATG TATCTGCTTT ATCATTGACA AATGATGAAT 660

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 193 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SENU2 5'

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

AGATGTTCCT GTTAATAACG AAAAGGCGTT GCAAAAGGCT GTTGCACATC AACCTGTGAG 60

CATTGCACTT GAAGCTGGTG GCAGAGACTT CCANCACTAC AAATCTGGTA TCTTCACTGG 120

AAAATGTGGT ACTGCAGTGG ATCATGGTGT AGTTATTGCT GGATATGGTA CTGAGAATNN 180

CATGGATTAT TGG 193

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 179 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SENU2 MIDDLE

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

TACAAGCACC AATACTGCGG AATGGGGTGA AAAAGCAGGG ACAACAGAGG ACGTGAATCG 60

ATACATTGAT TAACAAAACC TCATTTTTCC AGCAGAAAGG TGTAGAAACA GATGAATGCA 120

TTATCAAGAT TACAAGATGT TGATTAATGA TTTGTATATA TTGATATGTC ACTTGCTAT 179

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 200 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SENU2 3'

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

AGATGTTGAT TAATGATTTG TATATATTGG ATTATTGTTC AACTTTGTCT AATTATTCAG 60

TACATATTCC TTGTTTCTTA TTATGAAGAC TCCAAGTAAT GCTTTTTAGT CTTCCATCTG 120

TACTTGGTTT CAACATTAAT TAAAAAAAAG GACTATCTTC TGTACCTTTC ATTTAAAAAA 180

AAAAAAAAAA AAAAAAAAAA 200

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 965 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SENU2 PROPOSED

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

AGATGTTCCT GTTAATAACG AAAAGGCGTT GCAAAAGGCT GTTGCACATC AACCTGTGAG 60

CATTGCACTT GAAGCTGGTG GCAGAGACTT CCANCACTAC AAATCTGGTA TCTTCACTGG 120

AAAATGTGGT ACTGCAGTGG ATCATGGTGT AGTTATTGCT GGATATGGTA CTGAGAATNN 180

CATGGATTAT TGGATCGTTA GGAACTCATG GGGAGCTAAC TGCCGAGAGA ACGGCTACCT 240

CAGAGTCCAG CGTAACGTTT CCAGCTCTAG TGGCTTGTGT GGTTTAGCCA TAGAGCCTTC 300

ATATCCAGTA AAAACAGGAC CAAATCCTCC TAAACCCGCT CCATCTCCTC CATCTCCGGT 360

CAAGCCACCT ACAGAGTGTG ATGAATATTC TCAATGCGCT GTCGGCACCA CTTGCTGCTG 420

TATCCTTCAG TTCCGTAGGT CTTGCTTCTC TTGGGGATGC TGCCCACTTG AAGGAGCCAC 480

TTGCTGTGAA GACCACTACA GTTGCTGCCC ACACGACTAT CCTATCTGCA ATGTTCGTCA 540

AGGAACATGC TCAATGAGCA AGGGCAACCC ACTGGGAGTG AAGGCAATGA AGCGCATTCT 600

TGCACAACCT ATTGGGGCCT TCGGAAATGG AGGAAAGAAG AGCAGTTCTT GAATTCTACA 660

AGCACCAATA CTGCGGAATG GGGTGAAAAA GCAGGGACAA CAGAGGACGT GAATCGATAC 720

ATTGATTAAC AAAACCTCAT TTTTCCAGCA GAAAGGTGTA GAAACAGATG AATGCATTAT 780

CAAGATTACA AGATGTTGAT TAATGATTTG TATATATTGG ATTATTGTTC AACTTTGTCT 840

AATTATTCAG TACATATTCC TTGTTTCTTA TTATGAAGAC TCCAAGTAAT GCTTTTTAGT 900

CTTCCATCTG TACTTGGTTT CAACATTAAT TAAAAAAAAG GACTATCTTC TGTACCTTTC 960

ATTTC 965

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1388 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SENU3

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

CATTACAATA ATTACAATGT CACGTCTCTC GCTCGTATTG ATTCTCGTCG CCGGCCTTTT 60

CGCTACTGCA CTTGCCGGTC CGGCGACCTT CGCTGATAAG AATCCGATCA GGCAAGTCGT 120

ATTTCCCGAT GAGCTGGAGA ACGGGATTCT TCAAGTCGTC GGCCAGACTC GCAGTGCTCT 180

CTCCTTCGCT CGCTTTGCTA TCAGGCATCG GAAAAGGTAT GACTCCGTTG AAGAGATCAA 240

GCAAAGGTTT GAGATATTTT TGGACAATCT GAAGATGATC CGATCGCATA ACAGAAAAGG 300

ACTATCATAC AAACTCGGTA TCAATGAGTT TACCGACCTA ACATGGGATG AGTTCCGTAA 360

ACACAAGTTG GGGGCATCTC AAAACTGTTC TGCCACCACA AAGGGAAATC TCAAGCTCAC 420

TAACGTCGTT CTGCCAGAGA CGAAAGACTG GAGGAAAGAT GGTATTGTTA GCCCAGTGAA 480

GGCACAGGGC AAGTGCGGAT CTTGCTGGAC ATTCAGCACT ACTGGTGCAC TAGAGGCAGC 540

CTATGCCCAA GCATTTGGGA AGGGAATCTC TCTGTCAGAG CAGCAGCTTG TGGACTGTGC 600

TGGAGCTTTT AATAACTTTG GTTGCAATGG AGGGTTGCCT TCTCAAGCAT TTGAGTACAT 660

TAAATTCAAT GGTGGTCTTG ACACTGAAGA AGCATATCCA TACACCGGCA AGAATGGCAT 720

ATGTAAATTC TCACAAGCAA ATATTGGTGT CAAAGTCATC AGTTCTGTCA ATATTACCCT 780

GGGTGCTGAA TATGAACTGA AATACGCAGT TGCATTGGTT AGGCCTGTTA GTGTTGCTTT 840

TGAGGTGGTA AAAGGGTTCA AACAGTACAA GAGCGGAGTT TACGCCAGCA CTGAATGTGG 900

CGACACTCCC ATGGACGTAA ACCATGCTGT TCTTGCTGTG GGTTACGGTG TTGAAAATGG 960

TACTCCCTAC TGGCTCATAA AGAACTCATG GGGAGCAGAT TGGGGTGAGG ATGGATACTT 1020

CAAAATGGAG ATGGGAAAGA ATATGTGTGG TGTTGCAACT TGTGCATCCT ACCCAATCGT 1080

TGCCTAAGCT TTGGAGTTTT GTGAAAAAAA TCTGCATAAA TCCGTGTTGT CCCAGTTAAT 1140

GATTCAGCAG CAGCATTCAG GCTCCATTCT CAGATTTATA TTCTGAACAT GTATGGATTG 1200

TTATACATAT AAAAATGGTT TAGGCTACTT ATATGAAAGA AACAATAAGA TCAAAATATT 1260

TAGTTCACAG AGTTTATTAT GCAGGAAAAA ACCCTATGTA ATTTATACCA TTATAAGTAA 1320

TGAAATGGAA GAAGAATTTC TTATTGTAAA CATTGTTAAT AAGGTGTTGT CCTTAGTTCG 1380

TGTCTTTT 1388

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 523 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SENU4

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

GGCATCCCGA GCACAAAACT ATGCCAACTC AAGAGCTGGT GATTGTAACT TGATTCATTC 60

TGGTGCTGGG GAGAATCTTG CCAAGGGTGG TGGTGACTTC ACGGGGAGGG CAGCCGTGCA 120

ATTGTGGGTG TCCGAGAGGC CAAGCTATAA CTACGCTACC AACCAATGTG TTGGTGGAAA 180

AAAGTGTAGA CATTATACTC AAGTAGTCTG GCGCAACTCA GTCCGACTAG GTTGTGGTCG 240

GGCACGTTGC AACAACGGAT GGTGGTTCAT TTCTTGCAAC TATGATCCTG TAGGCAACTG 300

GATCGGACAA CGTCCTTACT AAAATGATCT ATACTTATGA CATGTTGCTA GTATTAAATA 360

AAATTCTCAT ATGAGACGTC GAGAAGTTAA AATTTAAGTT TGACATATGA ATCAAGTCAA 420

ACTCCTATCT AAAATATTAA GGGATTAAAT ATTGAACATC TATAATTATT ATTATTTCCC 480

TTTTGATGTT GCTAATATGA ATAATTCCAC ATACCATATG TTC 523

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 847 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SENU5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

ATTAAATTTA TGGAGAAGGT TAATTTTTTG AAAAATGGTG TATTAAGAAT TACCCCCAGG 60

TTTTCGATTT CGCTCCCACC GATGAAGAAC TTGTGGTACA ATACTTGAAG CGTAAAGTCT 120

TCTCTTTTCC TTTGCCAGCT TCTATTATCC CTGAAGTTGA AGTTTACAAA TCTGATCCTT 180

GGGATTTGCC AGGTGATATG GAGCAAGAAA AGTATTTTTT TAGCACTAAG GAAGTGAAGT 240

ATCCAAATGG GAATAGGTCA AATAGAGCAA CAAATTCAGG ATATTGGAAG GCTACTGGAA 300

TTGACAAGCA AATCATATTA AGGGGACGAC AACAACAACA ACAATTGATA GGATTGAAGA 360

AAACACTTGT CTTCTATAGA GGAAAATCTC CACATGGCTG TAGGACCAAT TGGATTATGC 420

ACGAATATCG TCTTGCCAAT CTCGAATCTA ACTATCATCC AATTCAGGGA AATTGGGTTA 480

TCTGTCGAAT TTTCTTGAAA AAAAGAGGCA ATACTAAAAA TAAGGAGGAA AACATGACAA 540

CACATGATGA GGTTAGAAAC AGAGAAATTG ATAAAAACTC GCCCGTTGTT TCAGTCAAAA 600

TGAGTTCTCG AGATTCTGAG GCATTGCTTC CGCGAATAGT GAACTGAAGA AGAAGGCATC 660

CATAATATTT TACGATTTTA TGGGGAGGAA TAATTCGAAT GGAGTTGCAG CTTCAACGTC 720

AAGTAGTGGA ATCACTGATT TGACTACTAC TAATGAAGAA TCTGATGATC ATGAAGAAAG 780

TACTAGTAGT TTTAATAATT TTACTACTTT TAAAAGAAAA ATTAATTAAT TAATCGTCGT 840

TAAAACG 847

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 212 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SEND31 5'

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

AAAAAAGAAT TTTTGATCAA AGATGGCACA ATACGGCAAT CAAGACCAAA TGCGCAAGAC 60

TGATGAATAT GGAAACCATG TCCAAGAAAC AGGAGTCTAT CAAGGTACCG GTACTGGCGG 120

TATGATGGGG GGCACGGGTA CTGGCGGTAT GATGGGGGGC ACTGGTGGAG AATATGGAAC 180

TCAAGGCATG GGTACTGGTA CTCATCACCA TG 212

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 180 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SEND31 3'

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

AATCTAATTA CGTACACTCT TGTGTTTAAA GTCGTGTAAA GTGCGGTGAC GCTATATGCA 60

TATATATATA TATTGGCGCC ATGCCCTGCC CCTTCTGTAT TTTTAAAACA AGAATATTGC 120

TTCCATGCTT GGAAAGCAAT GATCATATTG ATGTGAAAAA AAAAAAAAAA AAAAAAAAAA 180

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 721 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SEND31 PROPOSED

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

AAAAAAGAAT TTTTGATCAA AGATGGCACA ATACGGCAAT CAAGACCAAA TGCGCAAGAC 60

TGATGAATAT GGAAACCATG TCCAAGAAAC AGGAGTCTAT CAAGGTACCG GTACTGGCGG 120

TATGATGGGG GGCACGGGTA CTGGCGGTAT GATGGGGGGC ACTGGTGGAG AATATGGAAC 180

TCAAGGCATG GGTACTGGTA CTCATCACCA TGAGGGGCAA CAGCAGCTTC GTCGATCCGA 240

CAGCTCTAGC TCGTCGGAGG ATGATGGAGA AGGTGGGAGG AGAAAGAAGG GTTTGAAGGA 300

GAAGATAATG GAGAAGATGC CTGGACAACA TGAAGGTGAG TATGGACAAA CAACAGGTGA 360

AGAGAAGAAA GGAATGATGG ACAAAATCAA GGACAAGATC CCTGGGATGC ATTGAACACC 420

TTTGTTTTCA TCTCCATCTT ATCTTATGAA TAAATAAGGT AGTGCTTGAT TCTATTTCAT 480

GCACTAATTA GTAGTAATCG TTATGCCAGT AATTATCTAA TTACGTACAC TCTTGTGTTT 540

AAAGTCGTGT AAAGTGTGCT GACGCTATAT ACATGTGTGT GTATATTGGC GGCCATGCCC 600

TGCCCCTTCT GTATTTTTAA AACAAGAATA TTGCTTCCAT GCTTGGAAAG CAATGATCAT 660

GTTGATGTGT GTTATGGTGT CCCGTGTTTA ATGATGTTTT GGAAATAATA TAACTTGTGC 720

T 721

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 679 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: SEND32

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

AAGCAGACCA ATTTTTTCCC CACCAAACAA ATTCACACTC TGCTTTCTAT ATAAGCCTCA 60

CTCTTCATCC ACCAAAGCTC AAGAAACAGT CTGCTATAAC AATGGCTCAT CAGTCTCTTT 120

ACCTTCTCTT CCTCGTTGTC TTGTCATCAA TCAGGAGCCA TGTAATTCAA GTTGAGGCTC 180

GCCATCTTCT GGACGCAACT TTGCCAGATC TCCCCATTGG TCTCCCAAAA CCTGAGCTAC 240

CAACATTACC TATGCCCAAT CTTCCACTGC CGCAGCCTGG ATTGCCCACA CTACCTATGT 300

CCAATCTTCC ACTGCCGCAG CCTGGACTGC CCACATTACC TATGCCTAAT CTTCCTCAGC 360

CACAACTTCC AAACCCTCAG CTGCCACCTT TGCCCAAACC GGATTTGCCA TTACCTATGC 420

CACAACTTCC CAAACCTCAG CTACCTCTGC CACAGTTGCC AATACCATTG CCACCTCTGC 480

CAAGCCCTTA AGTACTAATG TTCCTTCAAT GATTGGGAAG TGTACTACGT GCTATCCAGG 540

ATGATTCGTT TGTATCATTA TTGTCGTATT TGTGAACCTG CTCATGTTAA TGTTGTTAAA 600

CTTTCGTATG TAATCTCTTA TTAGTAAGTC AATTGTTTGT CAGTTAACAT TATTAAATTA 660

CTTCTGTGAA TGATTCCTC 679

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 574 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SEND33

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

AGAAAAGAAA AGAATTGTAA AAAATGGCTA GTATTTCTGG TACAATGATT AGCACTTCTT 60

TCCTTCCAAG AAAGCCAGCT GTGACTAGCC TCAAAGCCAT ATCAAATGTT GGTGAAGCTT 120

TGTTTGGTCT TAAATCTGGT AGAAATGGGA GGATTACTTG CATGGCCAGT TACAAAGTGA 180

AGCTTATTAC ACCAGAAGGA CCTATTGAAT TTGAATGCCC AGATGATGTT TACATTCTTG 240

ACCAAGCTGA GGAAGAAGGA CATGACCTTC CTTACTCATG CAGGGCTGGT TCTTGCTCAT 300

CTTGTGCTGG AAAAGTTACT GCTGGAAGTG TTGATCAGTC TGATGGAAAC TTCCTTGATG 360

AGGACCAAGA AGCTGCTGGA TTTGTGCTTA CTTGTGTTGC TTACCCAAAG GGTGATGTTA 420

CCATTGAGAC TCACAAGGAG GAGGAGCTTA CTGCTTAAAT TACAACCATT TCCATTTTAA 480

TAAAAAACAA CACTTCATTT TGTTTCATGA GCATTTACTT TTCACATTTT CCCTCTATTG 540

TTATCACAAA AGTTGCTATC TAGTTATTTG TGAC 574

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 540 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SEND34

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

ATTGAGCCTA AANCCAACTT TCACTCTGGA GAAAATATCA GTGAAAGGGC TTCCATCACT 60

TACTAGATCT TCTTCTTCCT TCAAAGTTNT GGCTAGAGGT GTTAAGAAGC TTAAGACTGA 120

CAAACCCTAT GGAATTAATG GAAGCATGGC CTTGAGAGAT GGGGTTGATN CCTCAGGCAG 180

GAAGCCCAAG GGAAAGGGTG TGTACCAATA TGTTGACAAA TATGGAGCTA ATGTTGATGG 240

ATACAGTCCC ATCTACAACA CGGATGAATG GTCTCCAAGT GGTGATGTCT ATGTTGGAGG 300

TACCACTGGC TTANNCATAT GGGCGGTNAC CTTGCTTGGC ATTCTTGCAG GAGGTGCTCT 360

NCTTGTCTAC AACACAAGTN CTTTNNNGCA ATAGATGTTA TCCTTGTATT GTACTATTAA 420

GTTTAAGTTG TATTCCATGT TATCTTTTCA AAATATTTCT TGGCATCTTC ATTAAAATAT 480

TTCTTGGCAT CTTCATTAAT ATGTAATTGC TGTGATTTTA CTGTGGATAA TATTACATCT 540

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 524 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SEND35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

AACAAAGTGC AACCATTATC AACAGAGACA CAAAAAGAAA ACACAGAAAT GCAAAAATAC 60

ACAATCCTAG GAGCCAAGAA AATGGGAAAC AACAATGACA AATCAGTAGT TTGTCACAAA 120

CAAAACTATG CCTATGCAGT ATTTTATTGT CACAAAACAG AAACAACAGA GTCATACATG 180

GTATCATTAG TTGGTGTTGA TGGNACAAAA GTTAAGGCTG TGGCTGTTTG TCACAAAGGC 240

ACATCACAAT GGGACCCCAA AACATTTGGC TTTTAAAGTT CTTTAAGGTT ACCACCTGGG 300

NTCCCGTCCC CTGTTTTGTC ATTTCCCCCC ACAAGATCAC ATTGTTTGGG TCCCTAAGAA 360

CTAGAAAATA ATTAGATGAC ATATGTTTAA TTGTCTTCTA AAAATTTGGG TTGTTTTTAT 420

GTATTATATG TAATAATACA AAGTATGTTG TGTTTGTCTT ATACTTTTTG TCTTGTTGTA 480

TAATCATACT TTACTATACT AATAAATAAT ATATGTTCAC ATTT 524

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 474 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SENE71

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

CTCGAGGTTT GATCCTTGTC GTCCTGCTGA GCAGTACCCG ATTCCTTCTT GTGTCTTGAA 60

TGGAAGGCGT ACAAATTGTG TCATTCCGAA AGAAAACAAC TTCAAACAGG CAGGGGAGAG 120

GTACAGATCA TGGGAACCTG ACAGGCAAGA CAGATACATC AACAAATGGG TTGAGTCTTT 180

ATCCGATCCA CGAGTCACTC ATGAGATTCG CAGCATATGG ATATCATACT TGTCTCAGGC 240

TGACAAGTCC TGTGGTCAGA AGGTCGCTTC TCGTCTCACT GTGAAGCCTA CAATGTGAAA 300

AATCAATGAA AATAGTTGAA ATGGTTTCAA GCTGCAAATG TTGAAGGACT AATGCAAAAA 360

AACGTCCGCG TTGTGCTATA AACTGTACTT CTTTTTCAAT CGTAATGTTG TATTTTGTAT 420

CGAATTTCGA TGTCTTGTGT TTTTACTATA ATGATGTTGG AACATACAAC TGTG 474

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1442 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SEE1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

GAATTCGCGG CCGCTCCATT CTTCTCGCCT TACTCCCTCA CAGAACCCAG TAAAATATCG 60

CCAGTCCCGC CGTCGAGATG GCCCCACGCC GCCTGCTCGT CCTCGCCGTC GTCGCCCTCG 120

CGGCCACCGC CGCCGCGGCC AACTCCGGCT TCGCGGACTC CAACCCGATC CGCCCCGTCA 180

CCGACCGCGC GGCCTCCGCG CTCGAGTCCA CGGTCTTCGC CGCGCTCGGC CGCACCCGCG 240

ACGCGCTCCG CTTCGCACGC TTCGCCGTCA GGTACGGCAA GAGCTACGAG AGCGCGGCGG 300

AGGTCCACAA GCGGTTCAGG ATCTTCTCCG AGAGCCTCCA GCTGGTCCGC TCCACCAACC 360

GCAAAGGCCT CTCCTACCGC CTCGGCATCA ACCGCTTCGC GGACATGAGC TGGGAGGAGT 420

TCCGTGCGAC CCGGCTCGGT GCAGCCCAGA ACTGCTCCGC CACGCTTACC GGCAACCACC 480

GGATGCGCGC CGCCGCCGTT GCGCTGCCGG AGACGAAAGA CTGGAGGGAG GATGGGATTG 540

TGAGCCCAGT GAAAAACCAG GGCCACTGTG GATCATGCTG GACCTTCAGC ACTACTGGTG 600

CACTTGAGGC TGCATATACC CAGGCAACTG GCAAGCCCAT CTCTCTCTCT GAGCAACAGC 660

TTGTTGACTG TGGTTTTGCA TTCAACAATT TCGGATGCAA CGGAGGCCTT CCATCCCAGG 720

CCTTTGAATA CATCAAATAC AATGGTGGCC TTGACACTGA GGAATCTTAC CCTTACCAAG 780

GTGTCAATGG AATCTCCAAG TTTAAGAATG AGAATGTTGG AGTCAAGGTT TTGGACTCGG 840

TTAACATCAC CCTGGGTGCT GAGGATGAAC TGAAGGATGC TGTTGGTCTG GTTCGCCCAG 900

TTAGTGTTGC CTTCGAGGTG ATCACTGGTT TCAGGCTGTA CAAGAGCGGA GTTTACACTA 960

GCGACCATTG TGGAACTACA CCGATGGATG TGAACCACGC TGTTCTGGCT GTTGGCTACG 1020

GTGTCGAAGA TGGTGTACCC TACTGGCTCA TCAAGAACTC ATGGGGCGCT GACTGGGGTG 1080

ATGAGGGTTA CTTCAAGATG GAAATGGGCA AGAACATGTG CGGTGTTGCT ACGTGTGCAT 1140

CCTACCCTAT TGTCGCATGA GGCCCTTACG AAATGTTACA TGGTCTGTTT GGCATCAATA 1200

ATGCATGTTT AACCTGAGCT TGGCGATGGG TTATACAGAA CGGAAACTCT GTTTGTGAAT 1260

AGAAAATCAT GAAGGGAAGG AGTTGACCGG ATTCCTGCTT GTACGTCTCC CCGACTGTGT 1320

AGGTAGATTG TCAGTTGGGG TTCCGAAGTA CTCTACTCAT ACGTGTGTAT GACAGTTTAT 1380

TTATGAAACA AAATACGCAT TGATCGTTAT GGTCCAAAAA AAAAAAAAGC GGCCGCGAAT 1440

TC 1442

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 423 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SEE2 (T7 PRIMER)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

GAATTCGCGG GCGCTCCCGA GCCCTCCACC GGAGTATGAC ACTTGCTTGG GAGACCTGTA 60

TAGTGTTGCT TGGATGGAAG ACAGTGATTT CCACAATCTG CGAACTGAAT CTCTCAAGCA 120

GCAATACAAC TTGGTCAAGG ATAGGACAGC GGTTCAGGAT ACATTCAGCT ATGGCTCCCA 180

TGTGATGCAA TATGGTTCAT TGGAGTTGAA TGTTAAGCAT CTGTTTTCGT ACATTGGCAC 240

AAACCCTGCT AACGATGACA ACACGTCCAT AGAAGACAAC TCGTTGGCCA TCATTCTCAA 300

AGGCTGTTAA TCAGCGCGAC GCTGGACCTT GTCTTCTTCT GGCAGAAGTA CCGGAAATTG 360

GCAGACAGCT CACATGAGAA AAATGAAGCT CGGGGGGTTT GGCTTGAGGT GATGGCCACA 420

GGT 423

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 414 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SEE2 (T3 PRIMER)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

GAATTCCACC ACGGATAGTC CATTTCCCAA CAAGAATGGG GTAAAGTTTC ACATGAGCAT 60

CAGTCGGCTA CATAGAAATA GCAGTAACGC GTTCTTCATT TTTATGGAAT TACAAACTAC 120

ACATCTGCTA ACAAACTATA GGGAATATAT ACAGCAACGA CCAATACAAT TGCCAAATAA 180

CTTTCCGACT ATATATGTAC ACCGGAAATT GGGCATAGCT ATGTAATCTG GAACGGTGGA 240

ACAGCTATAG TAGTGGTATA AGTCTGCTTC ACCTATGGTT CAAGAACTAG GACCCCCCTG 300

AGGACTGGGT AATGTAAACC AAGTCCGTTG GGGNGGGAGC GGCTTGAAAT ATTTCCGCCT 360

CACCYTATGA TTCTTTAGGG CGCTTAAAAC CCTCGTGGGT AGAACTCCAG GGGT 414

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 181 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SEE3 5'

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

CGCGGTAGAN GGTATCGATA AGCTTGNATA TCGCAATNCG TCGGACGCTA TCAACCAGCA 60

TCACTGGCCT CAGGGGCACC GCCGAGTAAC GTGCAGTGCC ATGGTTGCTT CGGTCAACAT 120

GGGGNAACAC TTCTGGCACC GGTGTGCTCT TCACCAGGTA ACCCCAACAC CGGAGTAGAA 180

G 181

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 422 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SEE3 MIDDLE

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

GAATTCAGNC TGCTCCGCTA TCTCATCAGC CACCAGAGCT GCCCTGGGGN ATCTCAATCA 60

TTGTTCCAAC TTTGTACCCG ATAGTCTTGC CCACATTGGC GAACACTTCC TCAGCAACTT 120

GGCGGATAAG AGTCACTTGA TGCCCCAGTT CCTGTGGTGT TCCAACAAGA GGAACCATTA 180

TCTCTGGGAA CACTTGNAAC ACCCTGGTTG GTCATTGCTA TAGCAGCTTC AAAAATGGCC 240

CGGGCTTGCA TCTCTGTCAA TTCAGGGTAC GATATNCCAA GCCTGCACCC ACGGAAGCCA 300

AGCATCGGGT TTACTTCTGA AAGCTTTTCA ATTCGCGCGA GGGGATCCTC CTGGTTGGGT 360

TCCCGTCTTC AGCACATAAT CACTTACAAT GTCCTCGATG TTCCCCTTCC TNGAAGGGAC 420

TC 422

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 203 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SEE3 MIDDLE

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

GAATTCTTCT CCTCCGGAAC GAACGACCTG ACGCAGATGA CCTTTGGGTA CAGCAGGGNA 60

TGATGTGGGA AAGTTCATCC CCGNCTATCT TGCTCAGGGT CATTCTCCAA CATGACCCCT 120

TCGAGGNTCC TGGACCAGAG GGGTAGTGGG CGAGCTGGTG AAGCTTGCTA CAGAGCAGGG 180

GCCGNAAAGC TAGGCCTAAC TTG 203

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 262 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SEE3 3'

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

TTTAATCGAT AATTGATGAT GTATTGCATG AACAGAGTTT TGCANCCATG GNAGCAGTTT 60

GGCAAGTTTN ACATGCACTG GGGGAAAAAA AAGGACATGG GCATTGTTGC TTTCATGAGC 120

CACTGCCTAC TAGTTAATGT TCACATAGNC ATGGGCTCTG TAATAATACT GTTGGATCAC 180

CACATCCACC AGCAGCAGGC AATCCGGTTG CCAATGTAGG AGGCAGCCCT CCAGACAAGC 240

ACCTGAGCTG CAGCTAGCCT AG 262

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 191 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SEE4 5'

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

AATTCGCGGC CGCTCGAGAG ACTCCATTAT ATTATATTGC TCGATCTCTC CATCTGAACG 60

TACACACAGT CCAACACACA TGGCCGCCGC CGCCGCCGCC CTGGTGAGCA GCAACAGCCT 120

CCGCGCCCCG GCGGCCTTCT CCGTCGTCGT CCGCGCGGGG CTCCCGGNCG GAGGGTGTCG 180

TCGGAGTAGT G 191

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 304 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SEE4 3'

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

GAATTCGCGG CCGCTTTTTT TTTTGGTATG CATACGCATG AGGACCATGT ATAGTTAGTT 60

ATTATTACAT GGCGGCGTCG TTATTATTAT TATTACATGC ATGCAATGCA AGGAGAGCGT 120

GCGTACGTGG TGCATTAATT GCTGACTATA CTAGTACGAC TCGACGAGTG CATCCTCCTT 180

GTGCGTCTGG ATGACGAGGT CCGAGGTGGG GTAGGCGATG CAGGTGAGCA CAAAGCCCTC 240

GGCGACCTGG TCGTCGTCGA GGAAGCTCTG GTCGAACTGG TTGACGGAGC CGGAGACGAT 300

CTTG 304

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 94 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SEE5 3'

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

TTTTTTTTTT CACCTAGTAA TAATAATAAT AATACACATC CACTNGGTTT GGNTTGTCTT 60

GGTACAAAGG CGGGCACGGG CACGGGCTAT ACGT 94

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 270 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SEE6 5'

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

CAACCGGTCC AGCACTTTCA CGCTCAGCTC CAGCAATGGC TGCCTCCACC ATGGCGATCT 60

CCTCCACGGC GATGGCCGGC ACCCCCATCA AGGTGGGCTC CTTCGGCGAG GGCCGCATCA 120

CCATGCGCAA GACCGTGGGC AAGCCCAAGG TGGCGGNGTC CGGCAGACCC TGGTACGGGC 180

CCGACCGCGT CAAGTACCTC GGNCCCTTCT CCGGCGAGCC CCCGAGCTAC CTCACCGGCG 240

AGTTCCCCGG CGACTACGGN TGGGACACCG 270

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 353 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SEE6 3'

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

TTTTTTTAGA AGAAAAACAA ATTAATGGAT ATCAACAATT AACCAGCAGG AAACAAAAAC 60

CACCACTATT GCACAAACAT GGTTCATCTT AGTGTACAAC GCCAACTCAT CATCTCGACT 120

TGCCTATGCA ATGCAACGTG AAACAAAAGA CACACATGCA TCTCGCAGCA TCAATCAATC 180

CACGTACGTA CACCCTCTCC GATCCGAAAG ACAATGAAGT TGCATAGCAT AGCCGTTGGA 240

GCTTAGTTGN CGGGGACGAA GTTGGTGGCG TAGGNCCATG CGTTGTTGTT GACTGGGTCA 300

GCGATGTGGG TCAGCGAAGG TCTCGAGCGG GCCCTTGGCG GTGACGATGG CCT 353

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 301 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SEE7 5'

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

CAACTGCAAA GCAGCAAGCT CTACTCTTCT TCTGTACTGA ACGTGTGACT AGATAACAAT 60

AAGCGTGAAC CATGGCGGAC GAGTACGGCC GCAGCGGCTA CGGCAGGTCC GGCGCCGGCG 120

ACGACTACGA CAGCGGCTAC AACAGNAAGT CCGGCACTGA TGACTACGGC CGTGGCGAAG 180

GTGGCTACAA CAAGTCGGGC GGCGATGACG ACTACGGTCG CAGCGGCGGC GATGGGTACG 240

GCAGGTCCGG CGGCGACGAC TACGGNCGTN GCACCGGCGG CGGTGGCTAC AACAAGTCCC 300

G 301

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 425 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SEE7 3'

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

TTTTTTTTTA AGAGTGTCGA AATATATAGT GGTTTATTTC TTTCATTAAA AATAAAATAT 60

ACAGATGAAG AGAAAGCTTT TATTCTCAAA GCGTTGATAA AAAGCCGAGC GAAAACCACG 120

TGCACACATC GATCACCAAA CACGCGTAGG AAGTACCATG CACACACATC ATCAGTAACT 180

ACACATGATA TACATAGACG GACAATCAAA CCCCCGGCTG ATTCTTTTAT TGAGTTCGGT 240

CGGCCTCCAT GCATGCTCCT TCTGGTCCTG GGGAGGAGGC TACGTACGCA AGTAGATCGA 300

TCGAGCAGGA GGCCGATCAG CCGAAGAAGT GGNGTTCTTC TTCCCCTCGC CGGGGGGGGC 360

GGATTCTCCT CCTCCCCGGG GTCCCCGCGG GTNTTGGGCT CCTCTTGGCG NGGTGGTCGT 420

GGACG 425

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 388 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SEE8 (T3 PRIMER)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

CCCCAGGAAT GGGCAGCGGG CGTCGTCCTC CTTCCTCGGC GGCAAGACGC TGCTGAGACA 60

GGCCGAGGCG GCCGGCGTCG TTCGCCGTGC GCGCCGCGGG GACCCAACAG GCGATCTGGT 120

TCCCCGGCAG CAACCCTCCG CGTGGCTCGA GGCAGCTTCC CGGGATTCGT TTGTTCCCTG 180

GGGCTCGGAT CTGACCGGAG AGCTGGGNGG AACGTGAGGG GNGTGGTGCA TGCGGTGGGG 240

ATGTCGGGNG GGGATTCACC GAGTTCTGAC AGACGGTCCA NAACCTCTGT CACGCGGAAG 300

AGATCACAAC ACACCCTCAN ACAGTANCCA CGTGGCGGGC GCGTGGTNAT AAACCGAGGA 360

AACACCACTC CAGAAATACG ACAGTGTC 388

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 209 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SEE9 (T7 PRIMER)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

CCCAACACTA GTAGCGTAGC TGAGAGCCTG AGACGATGGC CGACTACTAC CACGGCGGCA 60

GGGNCATGTA CTCCAACACC GACGAGTGCT ACGACGCCGG CAGGCACGGC GGCAGCGTGA 120

GGGAGTACTC GNGCACCGAC GAGTACTACG ACAACGTGGA CGACCGCATG AGGAGGCCCG 180

CGTACGGCGC CGACGACTNC GGCTACGGC 209

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 390 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SEE9 (T3 PRIMER)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

GAATTCGCGG CCGCTCTTAA TTGCATGCAT GCATCATTAG ATATAGTAAC AGTGATGGAT 60

CGTTACACGC TGCAGAAGCA TGCGCTTAGT CGTTATTTGC AGACGATAAT AAGTAGATCG 120

ATGGAGTAGT GATCGAGCAA CACACACGTG ACGACGTTTG CTCTTATTTA CTTACACGCA 180

CGCACGTGTA CGGCGGCTAG GGGCCGTATA TATATCGAGT CCTGCATGCA TCTCGGATCG 240

GATGGGATGA TCAGTCGGTT TCAGTCGCAG TAGTAGGAGT AGCCCGNGCC CGTGCCCGTG 300

CCCGTGCGGC ACCCTGGTGC TCCTGCTGCC TGCTCTCGTA GGTGGGCCTC CTCCTTGCTC 360

GCGGGTGCTC GNGGGTACGC GTTGGCCGCC 390

(2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 347 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SEC1 (T3 PRIMER)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

GAATTCGCGG CCGCTTTTTT TTACCATGTG CAATACAGTT TCCGGTCTCA ATAAGATCAA 60

TACTAGTTTG TTGTTACAGG CAGCAGGCTG GCCATGCATG AATCATGACC GATGCAACGC 120

TTCAGGTTCC TTNACAACAC GCATGCATCT AGCTTTCCAC AGCAAACAAC TGGCGGCGGC 180

GGCAGCAGGT ATGCTGATGC ACCAACGTTC TAGTGGAACT TGAGGCTGGT GAGCACGTTG 240

TTGGTTGGAC GGGGGTCGGC GANGTGGTCG AGCAGGTTCT GGAACGGGCC AACGCCGGTG 300

GACGAGCCCC TGGATGAAGT AACCGAGGAT GGCGAGCATG GNGAGCC 347

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 151 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SEC2 5'

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

CTTGCCATGA ACACGTTCGC CAACGATATC ATCAGCCGCG CCGTGTCCGG NAAGTTCTTC 60

CGGGCGGAGG GNCGGAACAA GCTCTTCCGG GAGCTGGTGG AGGCCAACTC TNCTCTGTTC 120

GGAGGGTTCA ACCTGGAAGA CTACTTCCCG G 151

(2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 385 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SEC2 3'

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

TTTTTTTTAC AAAATAGCTT TTTTTATTAC TATATAAGTA AACACACACA TAAGATTAAC 60

ATAGTTTACA TGACCCGTAC GATAGCAACA CTCGGACATC TTTTCCTAGT ATAATAATAG 120

ATATATATAT ACATGGAATC CAAACTCCAA ACCATCTATA TCTCTGTAGA GTGTAGTTCA 180

CTTCACCATT GGCATGTGCA TTGTACGTAC GTACGTGCTC GTCCTCTACA NCCGCTGCAT 240

GCAGTTTTCC TTCGATCGTC AGCAGCTTGG AGGATGGAGT TGNCGAGCAG NTGCAGTAGG 300

TGCAGCAGTA GCAGGAAGCT TGGGAACAAG CATGAGCTTC TCCTTTGGAT GCACCGTGAG 360

TCCAAACAAC TCCGGCATGT CAACG 385

(2) INFORMATION FOR SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 375 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SED1 5'

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

GAATTCGCGG NCGCTCGCAG TCAAGATTTT CTGTTCATCT CTGGAACCAA GATGCGCACT 60

TATGCCAAAA CTGGAGAGAA TCCTCCAGAC GGTTTCATGT GCCCGGGTGG GTGGAAGGTT 120

CTTGTTGACT ACTACAACAG CCTGCAAGCT GAAGAAGCGA CTCCAGTCCC TGTATGAGGC 180

AAGCTACTAT TTGGACTAAG AGATTATTCA GTGGCGGGGG AAACATACAA GCAAGTGATC 240

AGATCATTTG GAGCTNTTTT CCTTATTGAT TCTTTAGGTT AGTAGTTGAT TGATATATGA 300

TCAGCATCTT TCNCGGATTG GGTGGTTTGG TCTTAAATTA TGAGACTCTG AAGCATGGNA 360

AAAAACTAAG GATCT 375

(2) INFORMATION FOR SEQ ID NO: 38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 388 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SED1 3'

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

GAATTCGCGG CCGCTTTTTT TTTTTTTTTT TTTTGTTGTT TTTTTTTTTT KTTTTTTTTT 60

TTTTATGNAC CCNTNCATCT TTTATTATTT AGTCCAACCG GNTTCGCTAC AGAACAATGG 120

CCATGCTGTK KGGGCTCAAC TTGNGCAGAT CTTTAGTTTT CCCATGCTTC AGAGTCTCAT 180

ATTTAAGACA AACAAACAAT CGAAAAGATC TTKATCATAT TTCAAATCAA CTACTTAACC 240

TAAAGRATCA ATAAGGGRAA AAAGSTCAAA ATGATCTKAT CACTTGCTTG TNTGTTCCCC 300

CNCCCATTGA ATAATCTCTT AGTCAAATAG GAGGTTGCCT CAATACAGGG AATTGGNGTC 360

GCTTCTTCAN CTTGGAGGTT GTGGGGGG 388

(2) INFORMATION FOR SEQ ID NO: 39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 296 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SED2 (T7 PRIMER)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

GCTAGCGCCT CCCTAGGTCT CGTTGTCAGC AATGGCCACC ACCGCTGCGT CCAGCCTCCT 60

CAAGTCCTCC TTCGCGGGCT CCCGGCTCCC TTCGGCCACG CGCACCACCA CCCCGTCGTC 120

CGTGGNCGTG GNCACCCCGN GCGGCGGGGG GGGGCCCATC CGCGNGTCCA TCTCCTCCCC 180

CAACCCGNCC TACGACCTGA CGTCCTTCTG GTTCAGCCCC ATCAAGGAGT CCATCGTGTC 240

CCGCGAGATG ACCCGGCGCT ACATGANGGA CATGATCACC CACGNCGACA CCGACG 296

CLAIMS

1. A method for producing plants having modified senescence characteristics which comprises transformation of plants with a DNA construct adapted to modify the expression of at least one senescence-related gene and subsequent selection of plants in which the senescence process is either inhibited or accelerated.

2. A DNA construct adapted to modify the expression of at least one senescence-related gene comprising a DNA sequence corresponding to at least part of a senescence-related gene preceded by a transcriptional initiation region operative in plants so that the construct can generate RNA in plant cells.

3. A DNA construct as claimed in claim 2 which generates antisense RNA.

4. A DNA construct as claimed in claim 2 in which the DNA sequence is a cDNA sequence.

5. A DNA construct as claimed in claim 2 in which the DNA sequence corresponds to at least part of the senescence-related sequence in the clone pSENU1, deposited at the National Collections of Industrial and Marine Bacteria under the accession number NCIMB 40571.

6. A DNA construct as claimed in claim 2 in which the DNA sequence corresponds to at least part of the senescence-related sequence in the clone pSENU2, deposited at the National Collections of Industrial and Marine Bacteria under the accession number NCIMB 40572.

7. A DNA construct as claimed in claim 2 in which the DNA sequence corresponds to at least part of the senescence-related sequence in the clone pSENU3, deposited at the National Collections of Industrial and Marine Bacteria under the accession number NCIMB 40573.

8. A DNA construct as claimed in claim 2 in which the DNA sequence corresponds to at least part of the senescence-related sequence in the clone pSENU4, deposited at the National Collections of Industrial and Marine Bacteria under the accession number NCIMB 40574.

9. A DNA construct as claimed in claim 2 in which the DNA sequence corresponds to at least part of the senescence-related sequence in the clone pSENU5, deposited at the National Collections of Industrial and Marine Bacteria under the accession number NCIMB 40575.

10. A DNA construct as claimed in claim 2 in which the DNA sequence corresponds to at least part of the senescence-related sequence in the clone pSEND31, deposited at the National Collections of Industrial and Marine Bacteria under the accession number NCIMB 40576.

11. A DNA construct as claimed in claim 2 in which the DNA sequence corresponds to at least part of the senescence-related sequence in the clone pSEND32, deposited at the National Collections of Industrial and Marine Bacteria under the accession number NCIMB 40577.

12. A DNA construct as claimed in claim 2 in which the DNA sequence corresponds to at least part of the senescence-related sequence in the clone pSEND33, deposited at the National Collections of Industrial and Marine Bacteria under the accession number NCIMB 40578.

13. A DNA construct as claimed in claim 2 in which the DNA sequence corresponds to at least part of the senescence-related sequence in the clone pSEND34, deposited at the National Collections of Industrial and Marine Bacteria under the accession number NCIMB 40579.

14. A DNA construct as claimed in claim 2 in which the DNA sequence corresponds to at least part of the senescence-related sequence in the clone pSEND35, deposited at the National Collections of Industrial and Marine Bacteria under the accession number NCIMB 40580.

15. A DNA construct as claimed in claim 2 in which the DNA sequence corresponds to at least part of the senescence-related sequence in the clone pSENE71, deposited at the National Collections of Industrial and Marine Bacteria under the accession number NCIMB 40581.

16. A DNA construct as claimed in claim 2 in which the DNA sequence corresponds to at least part of the senescence-related sequence in the clone SEE1, deposited at the National Collections of Industrial and Marine Bacteria under the accession number NCIMB 40582.

17. A DNA construct as claimed in claim 2 in which the DNA sequence corresponds to at least part of the senescence-related sequence in the clone SEE2, deposited at the National Collections of Industrial and Marine Bacteria under the accession number NCIMB 40584.

18. A DNA construct as claimed in claim 2 in which the DNA sequence corresponds to at least part of the senescence-related sequence in the clone SEE3, deposited at the National Collections of Industrial and Marine Bacteria under the accession number NCIMB 40570.

19. A DNA construct as claimed in claim 2 in which the DNA sequence corresponds to at least part of the senescence-related sequence in the clone SEE4, deposited at the National Collections of Industrial and Marine Bacteria under the accession number NCIMB 40583.

20. A DNA construct as claimed in claim 2 in which the DNA sequence corresponds to at least part of the senescence-related sequence in a clone selected from the group comprising SEE5, SEE6, SEE7, SEE8, SEE9, SEC1, SEC2, SED1 and SED2.

21. A DNA construct containing a sequence at least 20 bases in length ocurring in any one of the sequences shown as SEQ ID NO 1 to SEQ ID NO 39.

22. A DNA construct containing a sequence at least 20 bases in length ocurring in any one of the clones deposited at the National Collections of Industrial and Marine Bacteria under the accession numbers NCIMB 40570 to NCIMB 40584.

23. A method as claimed in claim 1 in which the DNA construct is a construct as claimed in any one of claims 2 to 22.

24. A plant cell containing a DNA construct as claimed in any one of claims 2 to 22.

25. A plant having modified senescence characteristics and derived from a cell as claimed in claim 24.

AMENDED CLAIMS

[received by the International Bureau on 11 January 1995 (11.01.95);
original claims 2,5-20 replaced by amended claim 1;
claims 1 and 23 replaced by amended claim 6;
claims 3,4,21,22,24 and 25 renumbered as claims 2,3,4,5,7 and 8 (2 pages)]

1. A DNA construct adapted to modify the expression of at least one senescence-related gene comprising a DNA sequence corresponding to at least part of a senescence-related gene preceded by a transcriptional initiation region operative in plants so that the construct can generate RNA in plant cells, wherein the DNA sequence corresponds to at least part of the senescence-related sequence in a clone selected from the group consisting of pSENU1, pSENU2, pSENU3, pSENU4, pSENU5, pSEND31, pSEND32, pSEND33, pSEND34, pSEND35, pSENE71, SEE1, SEE2, SEE3, SEE4, SEE5, SEE6, SEE7, SEE8, SEE9, SEC1, SEC2, SED1 and SED2.

2. A DNA construct as claimed in claim 1 which generates antisense RNA.

3. A DNA construct as claimed in claim 1 in which the DNA sequence is a cDNA sequence.

4. A DNA construct containing a sequence at least 20 bases in length ocurring in any one of the sequences shown as SEQ ID NO 1 to SEQ ID NO 39.

5. A DNA construct containing a sequence at least 20 bases in length ocurring in any one of the clones deposited at the National Collections of Industrial and Marine Bacteria under the accession numbers NCIMB 40570 to NCIMB 40584.

6. A method for producing plants having modified senescence characteristics which comprises transformation of plants with a DNA construct adapted to modify the expression of at least one senescence-related gene and subsequent selection of plants in which the senescence process is either inhibited or accelerated, wherein the DNA construct is a construct as claimed in any one of claims 1 to 5.

7. A plant cell containing a DNA construct as claimed in any one of claims 1 to 5.

8. A plant having modified senescence characteristics and derived from a cell as claimed in claim 7.

FIG. 1

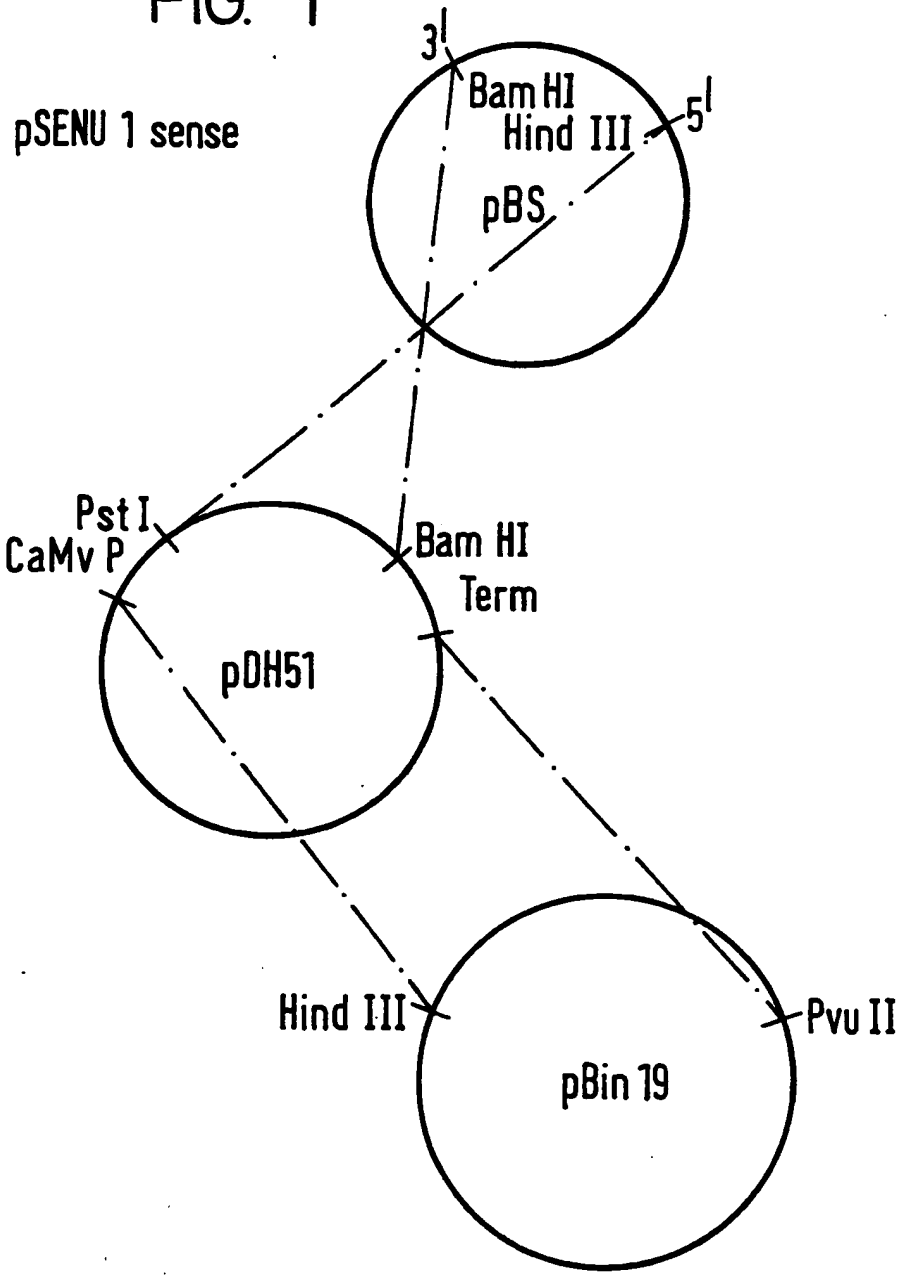

pSENU 1 sense
3'
Bam HI
Hind III
5'
pBS
Pst I
CaMv P
Bam HI
Term
pDH51
Hind III
Pvu II
pBin 19

FIG. 2

pSENU 1 antisense

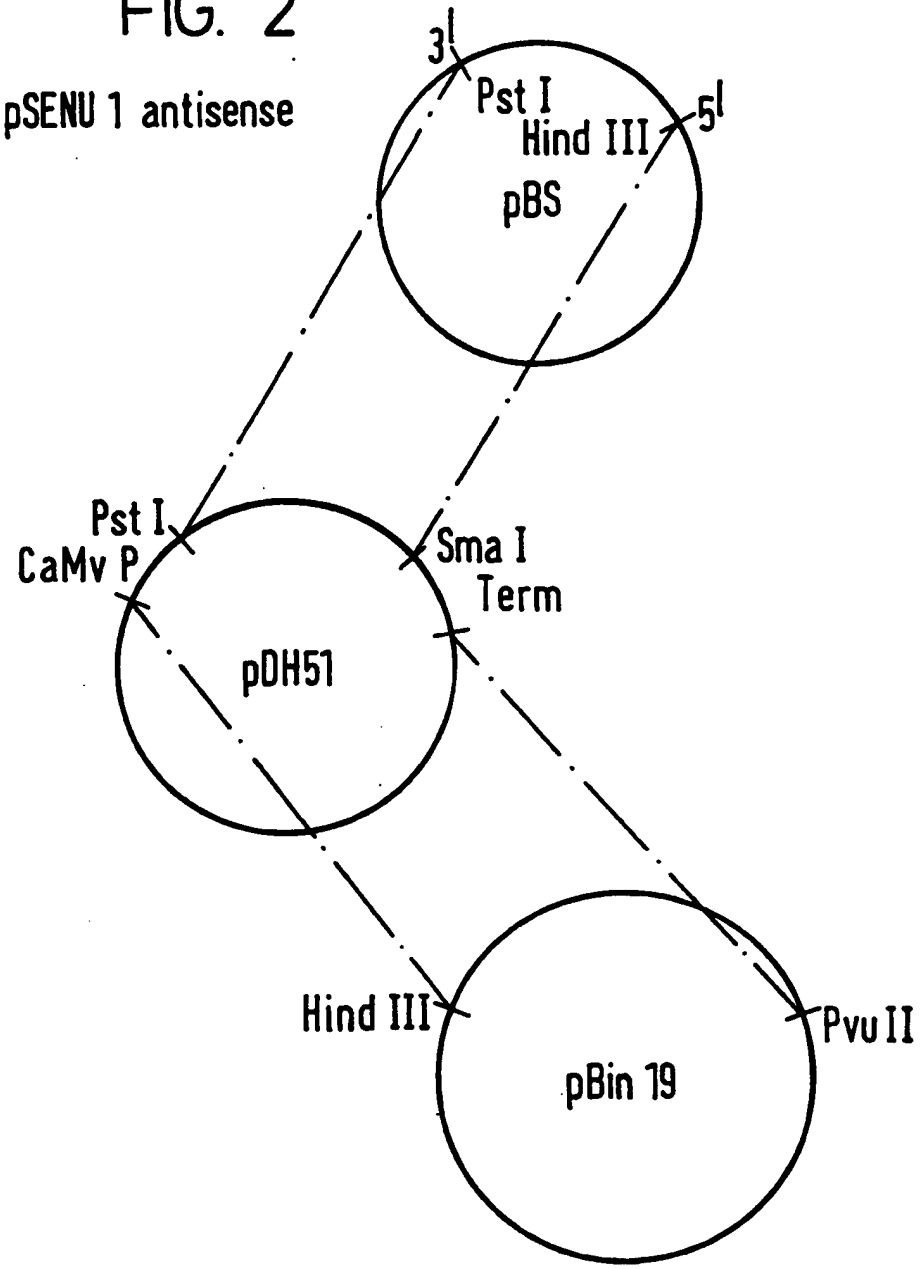


FIG. 3

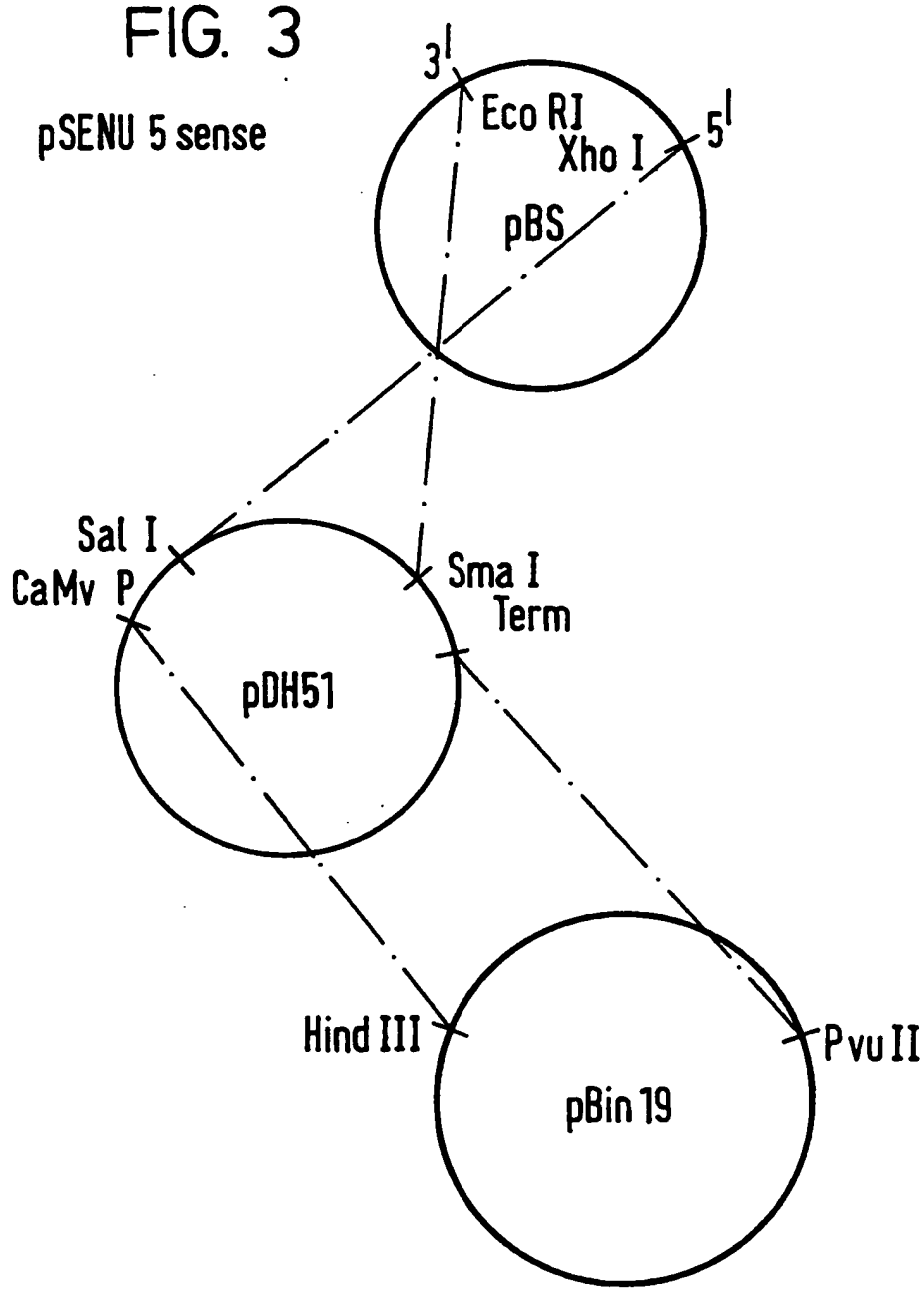
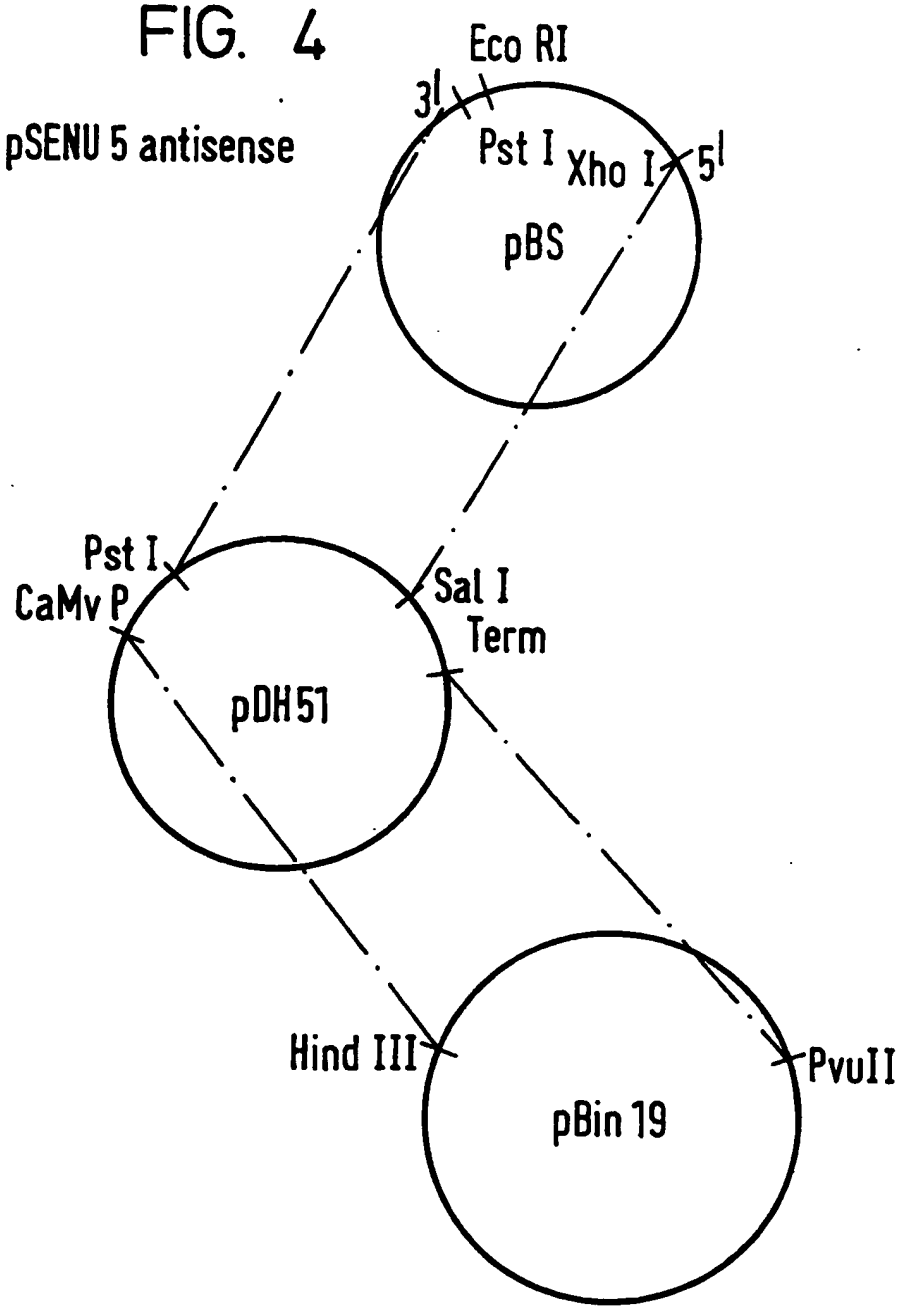

pSENU 5 sense
3'
Eco RI
Xho I
5'
pBS
Sal I
CaMv P
Sma I
Term
pDH51
Hind III
Pvu II
pBin 19

FIG. 4


pSENU 5 antisense
Eco RI
3'
Pst I
Xho I
5'
pBS
Pst I
CaMv P
Sal I
Term
pDH51
Hind III
PvuII
pBin 19

INTERNATIONAL SEARCH REPORT

Intern. al Application No
PCT/GB 94/01990

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/82 C12N15/29 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	THE PLANT JOURNAL, vol.3, no.3, 1993 pages 469 - 481 PICTON, S., ET AL. 'Altered fruit ripening and leaf senescence in tomatoes expressing an antisense ethylene-forming enzyme transgene' see page 476, right column - page 477, left column ---	1
X	PLANT PHYSIOLOGY, vol.96, 1991 pages 775 - 785 CHERRY, J. R., ET AL. 'Characterization of tobacco expressing functional oat phytochrome' see page 777, right column ---	1
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 December 1994	28. 12. 94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Maddox, A

INTERNATIONAL SEARCH REPORT

Intern al Application No
PCT/GB 94/01990

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 120, no. 1, 1994, Columbus, Ohio, US; abstract no. 1673, MICHAEL, M.Z., ET AL. 'Cloning of ethylene biosynthetic genes involved in petal senescence of carnation and petunia , and their antisense expression in transgenic plants' see abstract & CURRENT PLANT SCIENCE AND BIOTECHNOLOGY IN AGRICULTURE (CELLULAR AND MOLECULAR ASPECTS OF THE PLANT HORMONE ETHYLENE), vol.16, 1993 pages 298 - 303 & INTERNATIONAL SYMPOSIUM ON CELLULAR AND MOLECULAR ASPECTS OF BIOSYNTHESIS AND ACTION OF THE PLANT HORMONE ETHYLENE, AGEN, FRANCE, AUGUST 31-SEPTEMBER 4, 1992. ---	1
X	SCIENCE, vol.254, 18 October 1991 pages 437 - 439 OELLER, P.W., ET AL. 'Reversible inhibition of tomato fruit senescence by antisense RNA' see the whole document ---	1
X	THE PLANT JOURNAL, vol.4, no.1, July 1993 pages 179 - 189 DING, B., ET AL. 'Correlation between arrested secondary plasmodesmal development and onset of acclerated leaf senescence in yeast acid invertase transgenic plants' see the whole document ---	1
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol.88, August 1991, WASHINGTON US pages 7041 - 7045 SAITO, K., ET AL. 'Integration and expression of a rabbit liver cytochrome P-450 gene in transgenic Nicotiana tabacum' see the whole document ---	1
X	THE PLANT CELL, vol.3, no.7, July 1991 pages 647 - 656 SMART, C.M., ET AL. 'Delayed leaf senescence in tobacco plants transformed with tmr, a gene for cytokinin production in Agrobacterium.' see the whole document ---	1

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INTERNATIONAL SEARCH REPORT

Intern al Application No
PCT/GB 94/01990

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,89 09262 (MAX-PLANCK GESELLSCHAFT) 5 October 1992 see page 7, line 1 - line 7 see page 27 - page 28	1
X	WO,A,93 07272 (CALGENE) 15 April 1993 see page 13, line 15 - line 17	1
Y	WO,A,92 17596 (ICI) 15 October 1992 see page 4, line 13 - line 16	1
Y	PLANTA, vol.179, 1989 pages 73 - 80 DAVIES, K.M., ET AL. 'Identification of cDNA clones for tomato (Lycopersicon esculentum Mill.) mRNAs that accumulate during fruit ripening and leaf senescence in response to ethylene' see table 1	1
E	WO,A,94 21794 (ZENECA) 29 September 1994 see page 30 - page 33	1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB94/01990

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

see annex

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/GB94/01990

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

Claims searched incompletely: 1-25

In view of the extensive number of claimed DNA constructs as defined by claims 2-25 the search division considers that it is not economically reasonable to draw up a search report covering all the DNA constructs ' per se ',or for plants transformed with such DNA constructs or the methods to create said transgenic plants using said DNA , in accordance with PCT search guidelines, Chapter III paragraph 2.1. The search has been restricted to the subject matter of the methods of claim 1, resulting in the creation of transgenic plants with the functional features as defined therein, i.e. plants that have been modified in their senescence characteristics as a result of the intoduction of transgenes that are considered as senescence-related in that in general terms their expression results in said functional characteristics.The DNA constructs and the plants containing them as well as the methods for their creation as claimed in claims 2-25 have been searched in so far as relevant prior art would have been retrieved in the search for the method for claim 1 as defined above.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern al Application No
PCT/GB 94/01990

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-8909262	05-10-89	DE-A-	3810286	12-10-89
		AU-B-	633484	04-02-93
		AU-A-	3358989	16-10-89
		EP-A-	0334383	27-09-89
		EP-A-	0362349	11-04-90
WO-A-9307272	15-04-93	NONE		
WO-A-9217596	15-10-92	AU-A-	1434092	02-11-92
		BR-A-	9205814	28-06-94
		EP-A-	0618975	12-10-94
		JP-T-	6506110	14-07-94
WO-A-9421794	29-09-94	AU-B-	6262394	11-10-94